EVALUATION OF SPERMATOGENIC, APHRODISIAC AND ANTI-OXIDANT ACTIVITY OF CLASSICAL SIDDHA DRUG *THATHU VIRUTHI CHOORANAM* IN RODENTS

The dissertation submitted by

Dr. **D.MAHENDRAN**

Reg.No.321412108

Under the Guidance of

Prof. Dr.V.VELPANDIAN, M.D(S), Ph.D.,

Dissertation submitted to

THE TAMILNADU DR. MGR MEDICAL UNIVERSITY

CHENNAI-600032

In partial fulfilment of the requirements

for the award of the degree of

DOCTOR OF MEDICINE (SIDDHA)

BRANCH-II-GUNAPADAM

POST GRADUATE DEPARTMENT OF GUNAPADAM

GOVERNMENT SIDDHA MEDICAL COLLEGE

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GOVT. SIDDHA MEDICAL COLLEGE,

ARUMBAKKAM, CHENNAI-106.

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **"Evaluation of Spermatogenic, Aphrodisiac and Anti-oxidant Activity of classical Siddha drug "*Thathu Viruthi Chooranam"* in Rodents"** is a bonafide and genuine research work carried out by me under the guidance of **Dr.V.Velpandian M.D(S), Ph.D.,** Post Graduate Department of *Gunapadam*, Govt.Siddha Medical College, Arumbakkam, Chennai-106 and the dissertation has not formed the basis for the award of any Degree, Diploma, Fellowship or other similar title.

Date: **Signature of the Candidate**

Place: Chennai **Dr.D.Mahendran**

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ARUMBAKKAM, CHENNAI-106.

CERTIFICATE BY THE GUIDE

This is to certify that the dissertation entitled **"Evaluation of Spermatogenic, Aphrodisiac and Anti-oxidant Activity of classical Siddha drug "*Thathu Viruthi Chooranam*" in Rodents"** is submitted to the Tamilnadu Dr.M.G.R. Medical University in partial fulfillment of the requirements for the award of degree of M.D (Siddha) is the bonafide and genuine research work done by **Dr.D.Mahendran** under my supervision and guidance and the dissertation has not formed the basis for the award of any Degree, Diploma, Associateship, Fellowship or other similar title.

Date: **Signature of the Guide**

Place: Chennai

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ENDORSEMENT BY THE HOD AND PRINCIPAL OF THE INSTITUTION

This is to certify that the dissertation entitled **"Evaluation of Spermatogenic, Aphrodisiac and Anti-oxidant Activity of classical Siddha drug "*Thathu Viruthi Chooranam*" in Rodents"** is a bonafide work carried out by **Dr.D.Mahendran** under the guidance of **Dr.V.Velpandian M.D(s), Ph.D.,** Post graduate department of *Gunapadam*, Govt. Siddha Medical College, Chennai - 106.

Signature of the HOD **Signature of the Principal**

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ABBREVIATION

ACTH	**Adrenocorticotropic Hormone**
ALT	**Alanine Amino Transaminase**
AST	**Aspartate Amino Transferase**
ANOVA	**Analysis of variance**
ATP	**Adenosine Triphosphate**
AYUSH	**Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homoeopathy**
AZF	**Azoospermia Factor**
BHT	**Butylated Hydroxyl Toluene**
BSA	**Bovine Serum Albumin**
BUN	**Blood Urea Nitrogen**
CFTR	**Cystic Fibrosis Transmembrane Conductance Regulator**
CASA	**Computer Assisted Sperm Analysis**
CAT	**Catalase**
CMC	**Carboxy Methyl Cellulose**
CPCSEA	**Committee for the Purpose of Control and Supervision of Experiments on Animals**
CNS	**Central nervous system**
DBCP	**Dibromochloropropane**
DHT	**Dihydrotestosterone**

DMT	**Dimethyl tryptamine**
DOPA	**Dihydroxyphenylalanine**
DPPH	**1,1-Diphenyl-2-picrylhydrazyl**
EDTA	**Ethylenediaminetetraacetic acid**
FSH	**Follicle-Stimulating Hormone**
FTIR	**Fourier Transform Infrared (spectroscopy)**
GH	**Growth Hormone**
GIFT	**Gamete Intra Fallopian Transfer**
GIT	**Gastro Intestinal Tract**
GOT	**Glutamate Oxaloacetate Transaminase**
GP_x	**Glutathione Peroxidase**
GPT	**Glutamate Pyruvate Transaminase**
GnRH	**Gonadotropin Releasing Hormone**
GST	**Glutathione S-Transferase**
HAE	***Hygrophila auriculata* extract**
HPTLC	**High Performance Thin Layer Chromatography**
HZI	**Hemizona Index**
IAEC	**Institutional animal ethical committee**
IUI	**Intrauterine Insemination**
IV	**In Vitro Fertilization**
ICSI	**Intra-Cytoplasmic Sperm Injection**
ICPOES	**Inductively Coupled Plasma Optical Emission Spectroscopy**

IIT	**Indian Institute of Technology**
ISM	**Indian System of Medicine**
LH	**Leutinizing Hormone**
MEMP	**Methanol extract of *Mucuna pruriens***
MESA	**Micro Epididymal Sperm Aspiration**
MSH	**Melanocyte Stimulating Hormone**
NOAEL	**No Observed Adverse Effect Level**
OECD	**Organization for Economic Cooperation and Development**
PCV	**Packed cell volume**
POST	**Peritoneal Oozyte Sperm Transfer**
PESA	**Percutaneous Epididymal Sperm Aspiration**
RBC	**Red blood corpuscles**
SAIF	**Sophisticated Analytical Instrument Facility**
SPA	**Sperm Penetration Assay**
SEM	**Scanning Electron Microscope**
SHBG	**Sex Hormone –Binding Globulin**
SOD	**Superoxide Dismutase**
STD	**Sexual Transmitted Diseases**
SUZI	**Sub Zonal Sperm Injection (Directly into Ovum)**
TBARS	**Thiobarbituric Acid Reactive Substance**
TET	**Tubal Embryo Transfer**
TLC	**Thin Layer Chromatography**

TUFT	**Trans-Uterine Fallopian Transfer**
TVC	***Thathu Viruthi Chooranam***
WBC	**White blood corpuscles**
WHO	**World health organization**
XRD	**X-Ray Diffraction**
ZIFT	**Zygote Intra-Fallopian Transfer**

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INTRODUCTION

1. INTRODUCTION

The main function of the male reproductive system is to give men the ability to fertilise the woman's egg (ovum) by producing and delivering semen.

Male fertility requires the production of large number of normal spermatozoa by the testis through a complex process known as spermatogenesis. Spermatogenesis involves mitotic cell division, meiosis and the process of spermiogenesis. This is regulated by both endocrine and paracrine mechanisms. The endocrine stimulation of spermatogenesis involves both follicle stimulating hormone (FSH) and luteinizing hormone (LH)[1]. Any defect in these mechanism can result in the failure of the entire process and leads to production of defective spermatozoa and reduction of absence of sperm production.

Nearly 30 million couples in India suffer from infertility, in which the male is the affected partner is 40 - 50%. About 50 to 80 million couples suffer from infertility throughout the world. Male factors are considered to be the major cause of infertility in 30 % of cases[2].

The definition given by WHO for infertility, "Infertility is a disease of reproductive system defined by the failure to achieve a clinical pregnancy of 24 months or more of regular unprotected sexual intercourse".

WHO categorised 6 factors affecting male infertility

1. Congenital or acquired urogenital abnormalities
2. Urogenital tract infections
3. Increased scrotal temperature
4. Endocrine disturbances
5. Genetic abnormalities
6. Immunological factors

Though they categorized in clear way still 30-40% of male infertility cases were found to be idiopathic. These people show normal results in physical examination and endocrine investigations. However their semen analysis shows different picture like

- Decreased number of sperms - Oligozoospermia
- Decrease in sperm motility - Asthenozoospermia
- Other abnormal forms of sperm - Tetratozoospermia

In the year 2015, the WHO changed their guidelines for semen analysis for the diagnosis of infertile male[3]. man with reference values of greater than 20 million sperm, greater than 30% normal morphology and 50% progressive motility would be considered normal[4].

Male infertility can be caused by a number of health problems and medical treatments. Some of the medical causes are varicocele, high grade fever, urinary tract infection, STD, ejaculation issue, chromosomal defects, epididymitis, testicular injury, un descended tetis, orchitis, mumps, hormone imbalance, testosterone replacement therapy, long term anabolic steroid use, chemotherapy, excessive alcohol, smoking, certain anti-fungal medications and certain other medications can impair sperm production and decrease male fertility. Exposure to certain environmental elements like radiation or X-rays, heavy metals, industrial chemicals and exposure to over heat while handling electronic devices like lap-top can reduce sperm production or sperm function.

Male infertility can be stressful for both the partners resulting in emotional distress, marital problems and relationship difficulties. Distress in men is mainly emphasized with masculinity and fatherhood.

Treatment for male infertility vary according to underlying diseases and the degree of impairment of the male infertility. Testicular based male infertility tends to be resistant to medication. Usual approaches include, using the sperm for intrauterine insemination (IUI), In vitro fertilization (IVF) or IVF with intra cytoplasmic sperm injection (ICSI). Obstructive causes for post-testicular infertility can be overcome with either surgery or IVF-I CSI. Ejaculatory factors may be treatable by medication, or by IUI therapy or IVF. Vitamin E helps counter oxidative stress[5],which is associated with sperm DNA damage and reduced sperm motility[6].

A hormone-antioxidant combination may improve sperm count and motility[7]. Administration of lutenizing hormone (LH) and follicle-stimulating hormone (FSH) is

very effective in the treatment of male infertility due to hypogonadotropic hypogonodism[8].

Though androgens are absolutely essential for spermatogenesis and therefore male infertility, exogenous testosterone therapy has been found to be ineffective in benefiting men with low sperm count[9]. Moreover exogenous androgen therapy can actually impair or abolish male fertility by suppressing gonodotropin secretion from pituitary gland[10].

Siddha system of medicine is one of the oldest systems of medicine practiced in south India especially in Tamilnadu. The exponents of Siddha system of medicine are called *Siddhars*. They are the super human beings with high culture and intellectual abilities. They had tremendous powers by the way of meditations yoga practice and rejuvenation. They were spiritual scientists who explored and explained the way of nature and its relationship with man by their supernatural Siddhic powers.

The aim of Siddha medicine is to make body perfect, imperishable and to promote longevity. This is the first system to emphasis health as the perfect state of physical, mental, social, moral and spiritual component of human beings. This system mainly based "*ANDA PINDA" thathuvam* that means the relationship between the universe and human body. These two are interlinked through the five basic principles which are known as *"Panchaboothas".* The structural aspect of the human body is said to be *"Udal thathus"* (the physical components of body ie, *Saaram, Saenneer, Oon, Kolupu, Ennbu, Moolai, Sukkilam*) and functional unit of the human body is said to be *"Uyir thathus"* (the physiological units ie, *Vatham, Pitham* and *Kabam*). Functional co-operation of these two are essential for the maintenance of health.

ஆண் மலட்டின் குணம்

பார்க்கவே ஆண்மகனின் விந்து தானும்
பதமான தித்திப்புயில் லாததாலும்
ஏற்கவே சலமீதில் மிதந்ததாலும்
எழிலாக வுயிர்ப்பற்று யிருப்பதாலும்
சேர்க்கவே மூத்திரத்தில் நுரைதான் போலும்
செயலான கருவதுவும் தரிக்க மாட்டா
தீர்க்கவே யூகிமுனி சிகிச்சா சாரம்
தெளிவாகப் பாடிவைத்தார் திறமி தானே.

- யூகிமுனி

Treatments for infertility in modern medicines are mainly steroid based, hormone based therapies, resulting in hormonal imbalance and thus various health problems and also the treatments are very expensive comparatively the herbal treatment are highly effective.

Herbs have been used in traditional medicine for thousands of years to support the male reproductive system. Herbal remedies can stimulate healthy sperm production naturally. Most of these herbs are adaptogens which help to nourish the endocrine system so, all the hormones are working properly. Herbal remedies can be very effective in stimulating healthy sperm production, maintaining hormonal balance and supporting stronger erections to enhance male fertility and ensure conception. Herbal treatments are reliable and promotes overall healthy well being, since most of the herbal medicaments used for infertility are general tonics and alteratives.

The *Chooranam*, solid dosage form of medicaments, is meant for internal use. There are two types of *Chooranam*.

- Simple *Chooranam* - contains only one medicament.
- Compound *Chooranam* - contains two or more medicaments.

"*Thathu Viruthi Chooranam"* is a compound *Chooranam*, combination of the chooranam is as follows, *Nerunjil vaer (Tribulus terrestris), Neermulli vithai (Hygrophila auriculata), Nilappanai kizhangu (Curculigo orchioides), Poonaikkali vithai (Mucuna pruriens).* All the above four medicaments are ground with the juice of the whole plant *Nilappanai*. Then dried, powdered, sieved and bottled up.

Though medicinal plants and their parts have been used for the infertility treatment from ancient period, only few of them have been validated by scientific criteria. And also many new herbal medicines have been introduced in the world market to treat male infertility. All the drugs of male infertility medicine, "*Thathu Viruthi Chooranam*" have undergone various investigations for observing the action and mechanism of the phytochemicals with spermatogenesis activity. So, I consider my medicine "*Thathu Viruthi Chooranam*" as an unique preparation formulated by *Siddhars* for treating male infertility.

AIM AND OBJECTIVES

2. AIM AND OBJECTIVES

Aim

The aim of this thesis is to evaluate the Spermatogenic, Aphrodisiac and Anti - oxidant Activity of **"*Thathu Viruthi Chooranam*"** and to create the fingerprints to standardize this medicine with reference to the authentic drugs.

Objectives

The key objectives of the study are

- Having a collective review of the literature.
- Preparing the drug according to Siddha classical text.
- Subjecting the drug into physico-chemical standardization.
- Subjecting the drug into phyto-chemical standardization.
- Analyzing the drug chemically for detection of acid and basic radicals.
- Focusing the drug for analytical assessment through sophisticated analytical modern techniques like FTIR, ICPOES, SEM, XRD.
- Studying the toxicity profile of ***Thathu Viruthi Chooranam*** according to OECD guidelines.
- Evaluation the pharmacological study of the test drug ***Thathu Viruthi Chooranam*** through the following activities
- Spermatogenic, Aphrodisiac Activities in Wistar albino rats.
- Anti Oxidant - Through DPPH assay.
- Evaluation of microbial load for this formulation.
- Analyzing all the above study results to evaluate the benefits of ***Thathu Viruthi Chooranam.***

REVIEW OF LITERATURE

3. REVIEW OF LITERATURE

3.1. DRUG REVIEW

3.1.1. GUNAPADAM ASPECT

Nilapanai (Curculigo orchioides)

Alternative names[11]

Vaaraaki, Musali, Thiralaaram, Thirakathaaru, Thiranaraasan, Sakiyam, Thalamooli, Thalaiththaathu, Nilavizhumi, Naeyam, Kurathi, Sasiyam, Siththi.

Vernacular name

English : Black musale

Telugu : Naelatadi

Hindi : Musalikano

Kannada : Neladali

Parts used : Tuber, Root

Properties

Suvai : *Inippu*

Thanmai : *Thatpam*

Pirivu : *Inippu*

General character

“மேக வனல்தணியும் வெண்குட்டம் தான்விலகும்
போக மிகவுமுறும் பொற்கொடியே! போகாத
சூலைமே கங்களோடு துன்னுகரும் புள்ளியும்போஞ்
சால நிலப்பனைக்குத் தான்”

-அகத்தியர் குணவாகடம்

Actions

- Tonic
- Diuretic
- Astringent
- Carminative
- Emollient

Uses

It is used in the treatment of leucorrhoea, diabetes, eye diseases and male infertility.

Nerunjil (Tribulus terrestris)

Alternative names[11A]

Thirikandam, Thirikandakam, Thirithandam, Naerinjipudhum, Asuvasattiram, Suvathattam, Koekandam, Kaamarasi, Suvaathukandam, Kittiram, Koendam, Sutham.

Vernacular names

English : Small caltrops, Land caltrops

Telugu : Palleru

Sanskrit : Gokshura

Hindi : Gakhru

Malayalam : Nerunji

Part used : Whole plant

Properties

Suvai : *Thuvarppu, Inippu*

Thanmai : *Seetham*

Pirivu : *Inipu*

General properties

"நல்ல நெருஞ்சிலது நாளுங்கி ரிச்சிரத்தை
வல்ல சுரமனலை மாற்றுங்காண் - மெல்லியலே!
மாநிலத்தில் கல்லடைப்பும் வாங்காத நீர்க்கட்டும்
கூனுறுமெய் வாதமும்போக் கும்"

-அகத்தியர் குணவாகடம்

Actions

- Refrigerant
- Demulcent
- Diuretic
- Tonic
- Aphrodisiac
- Astringent

Uses

It is indicated as a best remedy for urinary tract disorders and male infertility.

Poonaikkaali (Mucuna pruriens)

Alternative names [11B]

Kandoothi, Markadi

Vernacular names

English : Common cowitch

Telugu : Pilliadagu

Malayalam : Choraivalli

Sanskrit : Atmagupta

Hindi : Kavach

Kannada : Nasugenne

Parts used : Seeds, Root

Properties

Suvai : *Thuvarppu*

Thanmai : *Thatpam*

Pirivu : *Inippu*

General properties

"தழுதளைநாற் றத்தோடு சாரிரத்தப் போக்கும்
பழுதுபுரி கின்றகரப் பானும் - அழுதேகுந்
தாலமிசை விந்துவுமாஞ் சாற்றற் கரும்பூனைக்
காலி விதையைக் கழறு"

-அகத்தியர் குணவாகடம்

Actions

- Astringent
- Nervine tonic
- Aphrodisiac
- Diuretic
- Vermifuge
- Irritant

Uses: It is used in the treatment of dropsy, leucorrhoea, male infertility, diarrhoea.

Neermulli (Hygrophila auriculata)

Alternative names[11C]

Mundagam, Kagandam, Dhurakathamoolam, Nithagam, Ikkuram.

Vernacular names

English : Long leaved barleria

Telugu : Nirugobbi

Malayalam : Vayalchulli

Sanskrit : Kokilaksha

Hindi : Talmakhana

Kannada : Kollavalike, Kalavankabija

Parts used : Seeds, Flower

Properties

Suvai : *Inippu, Siru Kaippu*

Thanmai : *Thatpam*

Pirivu : *Inippu*

General properties

‘‘விந்துவுமாம் தாதுவுமாம் மேகரோகந்தொலையும்
ஊந்து மதிசாரம் ஒழியுங்காண்-வந்துடலில்
ஏறியநீர் வீக்கம் இறங்கும் இளைப்புமறும்
கூறியநீர் முள்ளிவதைக் கு’’.

-அகத்தியர் குணவாகடம்

Actions

- Refrigerant
- Tonic
- Aphrodisiac
- Diuretic
- Demulcent

Uses: It is used in the treatment of leucorrhoea, dropsy, diarrhoea, male infertility.

3.1.2. BOTANICAL ASPECT

***Curculigo orchioides* (*Nilapanai*)**

Scientific classification[12]

Kingdom : Plantae

Order : Asparagales

Family : Hypoxidaceae

Genus : *Curculigo*

Species : *orchoides*

Synonyms

Sanskrit : Bhmitila

Assamese : Talmuli, Tailmuli

Bengali : Talmalu, Tallur

English : Black musale

Gujrati : Kalirnusali

Hindi : Syahmusali, Kalimusli

Kannada : Neltal, Neltathigodde, Nelatale, Nelatelegadde

Malayalam : Nilappenea

Marathi : Kali musali, Bhuimaddi

Oriya : Talamuli

Punjabi : Syah musali, Musali safed,

Tamil : Nilappanai

Telugu : Nel tadigadda

Urdu : Musali Siyah, Kali Musali

Distribution

Curculigo orchioides Gaertn. (Fam. Amaryllidaceae), a small herb, upto 30 cm high with tuberous root stock, occurring wild in sub-tropical Himalayas from Kumaon eastwards, ascending upto 1830 m in Khasi hills, Manipur and the Eastern

Ghats, also from Konkan southwards; drug is collected from two year old plants, washed well and cleared of rootlets, sliced and dried in shade.

Description

A herbaceous tuberous geophilous perennial with a short or elongate root-stock bearing several fleshy lateral roots; leaves simple, crowded on the short stem, sessile or short petioled with sheathing leaf bases, linear or linear-lanceolate ofter producing adventitious buds at the tips when in contact with soil; flowers bright yellow, the scape usually very short and hidden among the bases of the leaves underground, the lowest flowers bisexual, the upper male; fruits capsules; seed black, oblong, deeply grooved in wavy lines.

Constituents - Tannin, Resin, Sapogenin and Alkaloid.

Properties and Uses

The root-stocks are sweet, cooling, bitter, emollient, diuretic, aphrodisiac, alterant, appetiser, carminative, viriligenic, antipyretic and tonic, and are useful in vitiated conditions of *pitta* and *vatha*, Leucorrhoea, Haemorrhoids, Pruritus, Skin diseases, Asthma, Bronchitis, Jaundice, Diarrhea, Cuts and Wounds, Dyspepsia, Colic, Vomiting, Ophthalmia, Lumbago and Gonorrhea.

Tribulus terrestris (Nerunjil)

Scientific classification[12A]

Kingdom : Plantae

Order : Giraniales

Family : Zygophyllaceae

Genus : *Tribulus*

Species : *terrestris* Linn.

Synonyms

Assamese : Gokshura, Gukhurkata

Bengal : Gokshura, Gokhri

English : Caltrops root

Gujrati : Be tha gokharu, Nana gokharu, Mithogokharu

Hindi : Gokhru

Kannada : Sannanaggilu, Neggilamullu, Neggilu

Kashmiri : Michirkand, Pakhda

Malayalam : Nerinjil

Marathi : Sarate, Gokharu

Oriya : Gukhura, Gokhyura

Punjabi : Bhakhra, Gokhru

Tamil : Nerinjil, Nerunjil

Telugu : Palleruveru

Urdu : Khar-e-Khasak Khurd

Distribution

Throughout India, as a weed along roadsides and waste places.

Description

An annual or perennial, prostrate herb with many slender, spreading branches and silky-villous young parts; leaves abruptly simple, pinnate, opposite, leaflets almost sessile, rounded of oblique at the base, mucronate at the apex; flowers bright

yellow, solitary, pseudo-axillary or leaf-opposed; fruits, a 5-angled or winged spinous tuberculate woody schizocarp, separating into five cocci, each coccus having two long, stiff, sharp divaricate spines towards the distal half and two shorter ones nearer the base; seeds one or more in each coccus.

Constituents - Alkaloids and saponins.

Major chemical constituents

The major constituents of the fruit are steroidal saponins including gitonin, protodioscin (0.245%, tribulosaponins A and B, tribulosin and terrestrosins A–K, among others. Other constituents include alkaloids, tribulusamides A and B, and trace amounts of harman and norharman; and flavonols such as kaempferol, quercetin and rutin.

Properties and uses

The roots and fruits are sweet, cooling, Diuretic, Aphrodisiac, Emollient, Appetiser, Digestive, Anthelmintic, Expectorant, Anodyne, Anti-inflammatory, Alterant, Laxative, Cardiotonic, Styptic, Lithontriptic and Tonic. They are useful in Strangury, Dysuria, vitiated conditions of *Vatha* and *Pithta*, Renal and Vesical calculi, Anorexia, Dyspepsia, Helminthiasis, Cough, Asthma, Inflammations, Cardiopathy, Haemoptysis, Spermatorrhoea, Anaemia, Scabies, Ophthalmia and General weakness. The leaves are Astringent, Diuretic, Aphrodisiac, Depurative, Anthelmintic and Tonic. They are useful in Gonorrhoea, Gleet, Ulitis, Inflammation, Menorrhagia, Strangury, Leprosy, Skin diseases, Verminosis and General weakness. The seeds are Astringent, Strengthening and are useful in Epistaxis, Haemorrhages and Ulcerative Stomatitis. The ash of the whole plant is good for external application in Rheumatoid arthritis.

Mucuna pruriens (Poonaikkaali)

Scientific classification[12B]

Kingdom : Plantae

Order : Fabales

Family : Fabaceae

Genus : *Mucuna*

Species : *pruriens*

Synonyms

Bengali : Aalkushee, Alkusa

English : Cowhage, Cowitch

Gujrati : Kaucha, Kavach

Hindi : Kevanach, Kaunch, Khujanee

Kannada : Nasukunnee, Nasuganni, Nayisonanguballi

Malayalam : Shoriyanam, Naykkorana, Nayisonanguballi

Marathi : Khajkuhilee

Oriya : Baikhujnee

Punjabi : Aalkushee, Kavanch

Tamil : Punaik-Kalee, Punaikkalee, Punaippikukkam

Telugu : Piliyadugu, Pillee adugu

Urdu : Kaunch

Distribution

Throughout India among hedges and bushes

Description

A slender climbing annual with hairy branches; leaves trifoliate, leaflets broadly ovate, elliptic or rhomboid ovate, membranous, unequal at base, pubescent above and densely clothed with silvery grey hairs beneath; flowers purple, in axillary pendulous, -30 flowered racemes; fruits turgid pods, longitudinally ribbed, curved,

densely clothed with persistent pale brown or grey irritant bristles, seeds black, 4-6 per pod, ovoid.

Constituents - Choline

Chemical compounds

In addition to levodopa, it contains minor amounts of serotonin (5-HT), 5-HTP, nicotine, N, N-DMT (DMT), bufotenine and 5-MeO-DMT. As such, it could potentially have psychedelic effects, and it has purportedly been used in ayahuasca preparations. The mature seeds of the plant contain about 3.1–6.1% L-DOPA, with trace amounts of 5-hydroxy tryptamine (serotonin), nicotine, DMT-n-oxide, bufotenine, 5-MeO-DMT-n-oxide, and beta-carboline. One study using 36 samples of the seeds found no tryptamines present in them.

Properties and Uses

The roors are bitter, sweet, Thermogenic, Emollient, Stimulant, Purgative, Aphrodisiac, Diuretic, Emmenagogue, anthelmintic, febrifuge, diuretic and tonic. They are useful in vitiated conditions of *Vatha* and *Pittha*, Constipation, Nephropathy, Stangury, Dysmenorrhoea, Amenorrhoea, Elephantiasis, Dropsy, Neuropathy, Ulcers, Helminthiasis, Fever and Delirium. The leaves are Aphrodisiac, Anthelmintic and tonic, and are useful in Ulcers, Inflammation, Cephalagia and General debility. The seed are Astringent, Laxative, Anthelmintic, Aphrodisiac, Alexipharmic and Tonic. They are useful in Gonorrhea, Consumption, Sterility, vitiated conditions of *Vatta*, and General debility. The hairs and flowers are Vermifuge.

Hygrophila auriculata* (*Neermulli)

Scientific classification[12C]

Kingdom : Plantae

Order : Lamiales

Family : Acanthaceae

Genus : *Hygrophila*

Species : *auriculata*

Synonyms

Assamese : Kulekhara

Gujrati : Ekharo

Hindi : Talmakhana

Kannada : Nirmulli, Kolavulike, Kolavankae

Malayalam : Vayalculli, Nirchulli

Marathi : Talimakhana

Oriya : Koillekha, Koilrekha

Tamil : Nirmulle

Telugu : Talmakhana, Nerugobbi, Golmidi

Urdu : Talmakhana

Distribution

Throughout India, in plants, marshy places, rice-fields, margins of tanks and canals.

Description

An erect, hispid, semi woody, annual with numerous fasciculate sub-quadrangular stems; leaves in verticals of six at anode, 2 very large, upto 18cm long, bearing three straight, sharp yellow spines in each axil, hispid on both sides, lanceolate or oblanceolate, tapering at both ends, margins slightly serrate, flowers bluish purple or rose tint or whitish in axils of leaves, amidst spines, bracts and bracteoles, corolla bilipped, the upper 2-lobed and the lower 3-lobed; fruits linear oblong compressed capsules with 4-8 seeds on hard small retinacula, seeds flat or compressed, smooth with mucilaginous coating.

Constituents - Essential oil.

Major chemical constituents

The whole plant contains – Lupeol, stigmasterol, isoflavone glycoside, alkaloid and small quantities of uncharacterized bases.

The seed contains – Aasterol, Asteracanthine, Asteracanthicine, Aminoacids – Histidine, Lysine and Phenyl-alanine.

The fresh flowers contain – Apigenin-7-0-glucoside.

Properties and uses

The roots are sweet, sour, bitter, Refrigerant, Diuretic, Anti-inflammatory, Aphrodisiac and Tonic. They are useful in dropsy of chronic Bright's diseases, Inflammation, Ascities, Hyperdipsia, Vesical calculi, Strangury, Jaundice, Flatulence, Dysentery and vitiated conditions of *Vatha*. The leaves are sweet, sour, bitter, Oleaginous, Aphrodisiac, Anti-inflammatory, Stomachic, Ophthalmic and Tonic. They are useful in Jaundice, Dropsy, Rheumatism, Lumbago, Arthralgia, Anasarca, diseases of the Urogenital tract, Arthritis, Cough, vitiated conditions of *Pittha*, Gastropathy, Anaemia. The seeds are acrid, bitter, Refrigerant, Liver tonic, Aphrodisiac, Diuretic, Rejuvenating, Lithontriptic, Nervine tonic. They are useful in Gonorrhoea, Promoting sexual vigour and strength, arresting Abortion.

3.2. DISEASE REVIEW

3.2.1. Siddha aspect of the disease "*Aan maladu*"- Male infertility

சம்போக வாதம்

(Erectile Dysfunction)

"சம்போக வாதமது தையலார் சங்கமத்தின்
வம்போக வீழ்வதில் பிறக்கும் - அம்புந்
தளருமூர்ச் சிக்கும்நீர்த் தாகம் பலம்போய்
உளரும்பின் நோயாம் உரை"

-அகத்தியர் வைத்திய சிந்தாமணி 4000 பாடல் 145

Penis sluggishness, giddiness, increased thirst and loss of strength during coitus.

சுக்கிலவாதம்

(Premature Ejaculation)

"வாயுவாதம் காற்றினிடை வந்தால் அவயவங்கள்
பாயுங்கால் வலிக்கும் பண்ணுகுணம்-காயத்தின்
சுக்கிலக் காலந்திரத்திற் துன்னு துரிதமின்னும்
புக்கிநிறத் தாது கெட்டுப்போகும்".

-அகத்தியர் வைத்திய சிந்தாமணி 4000-பாடல் 144

Symptoms like earlier discharge of semen, semen dysfunction may occur.

மேட்ஷரிய வாதம்:

Ability and strength of the penis is decreased and there is pain, irritation, heaviness of the penis.

If the condition increases, then swelling of scrotum occurs.

-Roga Nirnaya Saaram 41

சுக்கிலத்தையடக்கினால்:

"சுக்கிலந் தனையடக்கின் சுரமுடனீர்க்கட்டாகும்
பக்கமாங் கைகால் சந்து பாரநோய் வழியிறங்கும்
மிக்கமார் நோயுண்டாகும் மிகுத்திடும் பிரமேகந்தான்
தக்கதோர் போதுமாகின் தரித்திடும் வாயுக் கூறே"[13]

-உடல்தத்துவம்

Discharge of semen is also one among physical urgent impulse. Obstruction of semen discharge results in Anuria, Fatigue, Involuntary discharge of semen, chest pain and palpitation.

"ஆண்மிகில் ஆணாம் பெண்மிகில் பெண்ணாம்
பூணிரண் டொத்துப் பொருந்தில் அலியாகுந்
தாண்மிகு மாகில் தரணி முழுதாளும்
பாவை மிக்கிடில் பாய்ந்ததும் இல்லையே"[14]

-திருமூலர் திருமந்திரம் 462

According to *Thirumoolar*, prolonged strength and stamina of the men partner during coitus may results in a baby with all superior characters and there is stoppage of semen discharge for the one who will have been fed up.

‘‘காயத்திலே மூன்று நாளிற் கலந்திட்டுக்
காயத்துட் டன்மன மாகுங் கலாவிந்து
நேயத்தே நின்றோர்க்கு நீங்கா விடாமையின்
மாயத்தே செல்வோர் மனத்தோடழியுமே’’[15].

-திருமூலர் திருமந்திரம் 1898

வாதம் வாழுமிடம்:

Vatha resides in places like reproductive tract lower abdomen, nerve bundles, muscles.

வளியின் இயற்கைப்பண்பு:

Provides vigour, strength and stamina to the sense organs and functioning of the 14 physical urgent impulse.

வளி செய்தொழில்:

Senselessness and inactivity of the organs.

வளி மிகுகுணம்:

Decrease in strength and stamina of the sense organs of the body.

நாடி நடை:

Vathapittham, *Pitthavatham*[16]

- Noi Naadal Noi Mudhal Naadal Thirattu

Maladu

According to T.V.Sambasivam pillai dictionary, A disease by which men or women are rendered incapable of producing child by reason of defective semen is termed as *Maladu* in Siddha[17].

Maladu rogam can be classified into two types:

1. *“Aan maladu”*
2. *“Pen maladu”*

The term “*maladan”* means a male with no issues. *Aan Maladu*: *Agathiar* suggest that *maladu* is only for male and not for female.

Yugimuni explains about *Aan maladu* in his treatise *Yugimuni Sikitcha Saaram.*

ஆண் மலட்டின் குணம்

"பார்க்கவே ஆண்மகனின் விந்து தானும்
பதமான தித்திப்புயில் லாத்தாலும்
ஏற்கவே சலமீதில் மிதந்த தாலும்
எழிலாக வுயிர்ப்பற்று யிருப்பதாலும்
சேர்க்கவே மூத்திரத்தில் நுரைதான் போலும்
செயலான கருவதுவும் தரிக்க மாட்டா
தீர்க்கவே யூகிமுனி சிகிச்சா சாரம்
தெளிவாக பாடி வைத்தார் திறமி தானே[18]".

-மகளிர் மருத்துவம்

According to *Yugimuni*, a person with semen of following qualities to infertile

- Lack of sweetness
- Buoyancy on water
- Absence of virility/viability
- And frothy micturition

Semen formation

According to *Thirumoolar Thirumanthiram*, at the time of *sirushti*, *kundali* appears in semen. Semen functions as God's *kiriyashakthi* during formation of embryo.

விந்துவின் தோற்றம்

"இரத முதலான ஏழ்தாது மூன்றின்
உரிய தினத்தின் ஒருபுற் பனிபோல்
அரியதுளி விந்து வாகுமேழ் மூன்றின்
மருவிய விந்து வளருங்கா யதிதிலே"[19]

-திருமந்திரம் 1897

இரதமாகிய சாரம் முதலாகச் சொல்லப்படும் ஏழ் முதற் பொருள்களால் ஆக்கப்பட்டதிவ்வுடல். இவ்வேழு உடற் கட்டுகளுள் சாரம், செந்நீர், வெண்ணீர் ஆகிய மூன்றும் ஓர் நாள் ஒரு புற்பனி போல் திரளும், இத்திரட்சியே விந்து எனப்படும். இவ்விந்து ஏழ் மூன்றாகிய இருபத்தொரு நாட்கள் வரை வளரும்.

In the above verses states the significance of sperm. In modern correlation spermatogenesis the process by which the male gamete called spermatozoa are formed by various stages like proliferation, growth, maturation, transformation.

As per the *Thirumoolar Thirumanthiram* it has been described that 6400 drops of Blood make one drop of *Vindhu* (Example: 80 drops of blood make one drop of white corpuscle and 80 drops of white corpuscle make one drop of *Vindhu*)[20].

Thus 80 x 80 = 6400 drops of blood makes one drop of *Vindhu*.

If extensive loss of *Vindhu* occurs in one human body naturally it will reflect on blood cells.

According to *Agathiyar Vaidya Valladi* – 600[21] – The *Vindhu* (Semen) is chiefly constituted by the Fire (*Vanni)* and Air (*Vayu*) elements.

Configuration of semen

Based on Siddha principles the configuration of semen is eighty drops of blood is equal to one drop of semen. Therefore wasting a drop of semen is equal to wasting six thousand four hundred drops of blood. Siddhars revealed their approach to a infertile patient in different aspects. *Dhanwanthri* explains a disease associated more with defective semen: *sukkilapitham*.

சுக்கில பித்தம்

“தக்கதாங் கர்ப்பந் தன்னைத் தவிர்த்திடுங் கனவு தன்னில்
சக்கிடுஞ் சுக்கிலத்தை சுக்கில நாளங் காந்தும்
மிக்கசுகிலம் போல்நீரில் வெள்ளையுங் காணுங்கண்டாற்
சுக்கிலபித்தம்மென்றே சொல்லினர் சுருதிவல்லோர்”[22]

-தன்வந்திரி வைத்தியம்

Sukkilapitham is characterised by

- The semen is incapable to impregnate women
- Nocturnal emission
- Burning sensation in ejaculatory ducts
- When a drop of semen is poured on water it will float with white colour.

The views of *Yugimuni* and *Dhanwanthri* synchronise in one thing that a semen which floats on a water surface will be incapable for fertility.

According to *Theraiyar Yamagam*[23]

Theraiyar explains the different qualities of semen. He compared the physical nature of semen with known things proposed the ratings for the quality of semen.

Table No.1. Nature and Inference of the sperm

Nature of semen	**Inference**
White and akin to the butter	Excellent
White akin to curd	Very good
White and akin to milk	Good
White and akin to buttermilk	Fair
Akin to honey in colour and consistency	Average
Akin to ghee in colour and weight	Poor
Akin to toddy in colour and thickness	Very poor
Akin to water	Very bad

Siddhars thought about the basic units of reproduction- semen and ovum, crossed the limits of procreation and waved towards attaining their spiritual target. In Siddha, *sukkilam* is considered as *sivam* and *naadham* is considered as *sakthi*. By which *karu* is considered as *sathasivam*.

According to *Agathiyar*, significance of sperm

“விந்துநிலை யறிந்து பாயுங்காலம்
வேதாந்த நாதமதுக்கு இருண்டாகி
சொந்தமுடநே இருண்டுமே நியுமாகி
சோதிமனி யானதுவிம்பி றையுமாகி
வட்னமதப் பிறையதுவும் வட்டமாகி
வட்டமதி ரண்டுருவாய் மண்ணுமாகி
அந்தமுள்ள மண்ணுதறி உப்பாய் நின்றே
யாதியென்ற பொருளான பிண்டமாச்சே”[24]

-அகத்தியர்

After the penetration of the sperm into the ovum, the sperm head fuse with the oocyte to form a single cell. Then it undergoes several stages of cell division and finally form the embryo.

Sage S*ivavaakiyar* and sage *Thirumoolar* explains the role of semen after entering uterus.

சுக்கில குணம்

"உண்மையான சுக்கில முபாயமா மிருந்ததும்
வெண்மையாகி நீரிலே விரைந்துனீர் தானதும்
தண்மையான காயமே தரித்துருவமானதும்
தெண்மையான ஞானிக தெளிந்துரைக்க வேணுமே"

-சிவவாக்கியர்

At the time of copulation, the semen is ejaculated. The prostatic fluid gives the semen a milky appearance. In the early minutes after ejaculation, the sperm remains immotile, possibly because of the coagulum. As the coagulum dissolves the sperm becomes highly motile.

"உன்னிய கர்ப்பக் குழியாம் வெளியிலே
பன்னிய நாதம் பகர்ந்த பிருத்திவி
வன்னியும் வாயுவும் மாயுருஞ் சுக்கிலம்
மன்னைய சமனாய் வளடர்க்கு முதகமே
விழுந்தது இலிங்கம் விரிந்தது யோனி
ஒழிந்த முதல் ஐந்தும் ஈரைந்தோடு ஏறிப்
பொழிந்த புனல்பூதம் போற்றும் கரணம்
ஒழிந்த நுதல் உச்சி உள்ளே உளித்ததே"[25]

-திருமூலர்

The ovum consists of element earth whereas the sperm consists of elements fire and air. The uterine wall which nourishes it has water and the uterine cavity is of the elemental space. Therefore all the five elements combine and create the foetus.

"வேர்க்கவே வேலிபோல் வளைந்து காக்கும்
விந்துவுடன் பிராணவாயு விளக்களாமே"[26].

-யூகிமுனி

Abanan stays outside and the *pranan* goes along with spermatozoa and bisects the size of the zygote. *Udhanan* helps in the growth of an embryo.

According to *Theraiyar*

- Food nourishes *saaram* or essence on the first day
- It then nourishes blood on the second day
- It then nourishes muscles on the third day
- It then nourishes adipose tissue on the fourth day
- It then nourishes bone on the fifth day
- It then nourishes bone marrow on the sixth day
- It then nourishes semen on the seventh day
- Concepts about formation of embryo by semen

The variations of the *Sukkilam* – physical constituents excess and decreased

- Excess *Sukkilam* causes love and lust towards women and also urinary calculi.
- Decreased *Sukkilam* causes failure in reproduction, pain in the genitalia etc.

Sex variation in foetus

At the time of copulation if the male dominates then the foetus will be male and if the female dominates then it would be female foetus. If the both are in equal domination, the child would be eunuch. Disease associated more with defective semen.

1. *Sukkilavaatham*

Symptoms of *Sukkilavaatham*: emaciation, constipation, oliguria, bleeding from the nose, phlegm accumulation due to increased *kabam*, breathlessness, loss of taste, abnormal semen[27].

2. *Sukkilapittham*

Sukkilapitham is characterised by the semen which is incapable to impregnate women, nocturnal emission, burning sensation in ejaculatory ducts. When a drop of semen is poured on water it will float with white colour[27A].

3. *Vali Azhal Suram*

Characterised by fever, rigor, sneezing, restlessness, *thathunattam*, nausea.[27B]

4. *Karumpanasai ammai*

According to "*Agathiyar vaisoori nool*" *Karumpanasai ammai"* will affect the semen and can make the patient infertile[27C].

5. *Iya maladu*

Indhiriya nashtam is one of the symptoms of *iya maladu* along with vomiting, sneezing, expectoration, hip pain etc.

6. *Perumanjal kaamalai*

This disease is characterized by *thaathunattam*, yellowish discolouration of face, eye, tongue, skin, loss of appetite, dyspnoea etc., these diseases mentioned above, if not properly treated may lead on to *Aan maladu*[27D].

"வெல்லும் புவியில் விளங்கிய தாபரம்
புல்லிடுஞ் சங்கமம் பொறியிடும் விந்தே
விந்தினாலல்லோ மேதினி யாச்சுது
செந்துக்களெஞ்லார்ஞ் சிவமயமாச்சுது
வந்திடும் நாதம் மௌனம் கலந்தது
சிந்தையிலுள்ளத் தெளிவாகச் செப்புமே"

-நோய் நாடல் நோய் முதனாடல் திரட்டு[28]

Siddhar Therayar says that the life in world originated from semen enters the uterus and fertilises the ovum.

"ஆண்மையென்று மங்கையர்கள் பூக்குங்காலம்
அன்று முதல் பதினாலு நாளும் அந்தத்
தான்மையன்றிப் பதினாறு இதமாய் நின்ற
தாமரைபோல் மலர்ந்திருக்குஞ் சாற்றக் கேளு
காண்மையன்றி தினமொன்று இதழ் தானென்று
கருவான கருக்குழிதான் இந் நாட்டுக்குள்ளே
பான்மையென்ற விந்தங்கே யூறும்போது
பாயுமப்பா வன்னியோடு வாயு தானே!"

-மருத்துவாங்க சுருக்கம்[29]

According To *Agathiyar Vaidya Valladi* 600- The *Vindhu* (Semen) is chiefly constituted by the fire (*Vanni*) and air (*Vayu*) elements.

According to *Thiruvalluva nayanar gnanavettiyan* – 1500[30]

Thiruvalluva nayanar clearly explained that the hormonal influence and brain stem is characterized with the following features.

"விந்து குடியிருந்த திருநாட்டை விட்டேன்
மாறுகின்ற கத்திரிக்கோல் பட்டந்தன்னில்
விந்து நின்று விளங்கு நதி மையத்துள்ளே
விளங்கு சுவாதிஷ்டான வெளியிலேதான்"

- திருவள்ளுவ நாயனார்

Spermatogenesis is stimulated by commands from the cerebral cortex. Decussating of the fibres in the brain stem explain this. Spermatogenesis is controlled by pituitary gland and hypothalamus.

"சுக்கிலந் தனையடக்கின்
சுரமுடனீர்க் கட்டாகும்
பக்கமாய் கை கால் சந்து
பாரநோய் வழியிறங்கும்
மிக்க மார் நோயுண்டாகும்
மிகுந்திடும் பிரமேகந்தான்
தக்கதோர் போதுமாகின்
தரித்திடும் வாயுக் கூறே"[31]

-உடல் தத்துவம்

3.2.2. Modern aspect of the disease/Male infertility

Infertility

The WHO defines infertility as a disease if the reproductive system that impairs the body's ability to perform the basic function of reproduction. The husband is responsible for the infertility of approximately one half of all childless marriages. There is usually defective development of the germinal epithelium in the somniferous tubules, with oligospermia or azoospermia[32]. Although conceiving a child may seem to be simple and natural, the physiological process is quite complicated and depends on the proper function of many factors, including the following,

- Production of healthy sperm by the man
- Production of healthy ovum by the woman
- Unblocked fallopian tubes that allow the sperm to reach the ovum
- The sperm's ability to fertilize the ovum
- The ability of the fertilized ovum to become implanted in the uterus

Male Infertility - Definition

Male factor infertility is said to be present when a couple fails to achieve pregnancy after one year of unprotected coitus and a problem is identified in the male partner.

Causes of infertility in men can be explained by deficiencies in ejaculate volume causing low sperm production (oligospermia), poor sperm motility (asthenospermia), abnormal morphology (teratospermia), and abnormal sperm function or by preventing sperm transport to vagina.

Classification

Primary Infertility

This is when the man has never impregnated women.

Secondary Infertility

The inability to become pregnant, or to carry a pregnancy to term, following the birth of one or more biological children. The birth of the first child does not involve any assisted reproductive technologies or fertility medication.

Structures of Male Genital Organ

Male reproductive system

The male reproductive system consists of the primary reproductive organs, the testis and the secondary reproductive organs, which includes,

- Scrotum
- Epididymis
- Vas deferens
- Seminal vesicles
- Prostate gland
- Urethra
- Bulbo-urethral glands and
- Penis

The testis in which the sperm cells develop are located outside the body cavity in the scrotum where the temperature is lower.

The formation of the male gamete from germ cell to mature spermatozoon takes approximately 70 days and proceeds within the confines of the seminiferous tubules[33].

Sperm cells are transported from the testes to the epididymis, which lies on the external surface of each testis and then through the ductus deferens into the prostate.

Just before the ductus deferens enters the prostate gland, the ductus deferens increases in diameter to become the ampulla of the ductus deferens.

A short duct of the seminal vesicle joins the ampulla of the ductus deferens to form the ejaculatory duct at the prostate, which then projects through the prostate gland and empties into the urethra, with in the prostate gland.

The urethra exists from the pelvis and passes through the penis to the outside of the body.

Physiology

- Spermatogenesis
- Performance of the male sexual act
- Regulation of male reproductive functions by the various hormones.

Spermatogenesis

Spermatogenesis occurs in all the seminiferous tubules during active sexual life as the result of stimulation by anterior pituitary gonadotropic hormones. The Process of spermatogenesis takes approximately 60-70 days from the beginning of the differentiation of the spermatocyte to a completion of the motile sperm.

When the sperm leave the testis they are relatively immature and have a poor capacity to fertilize. The transport of the sperm through the epididymis to the ejaculatory duct requires an additional 12 to 21 days.

During passage through the epididymis the spermatozoa has the following features,

- Maturation takes place
- Sustained motility
- Modification of nuclear chromatin and tail organelles
- Loaf of spermatid cytoplasm

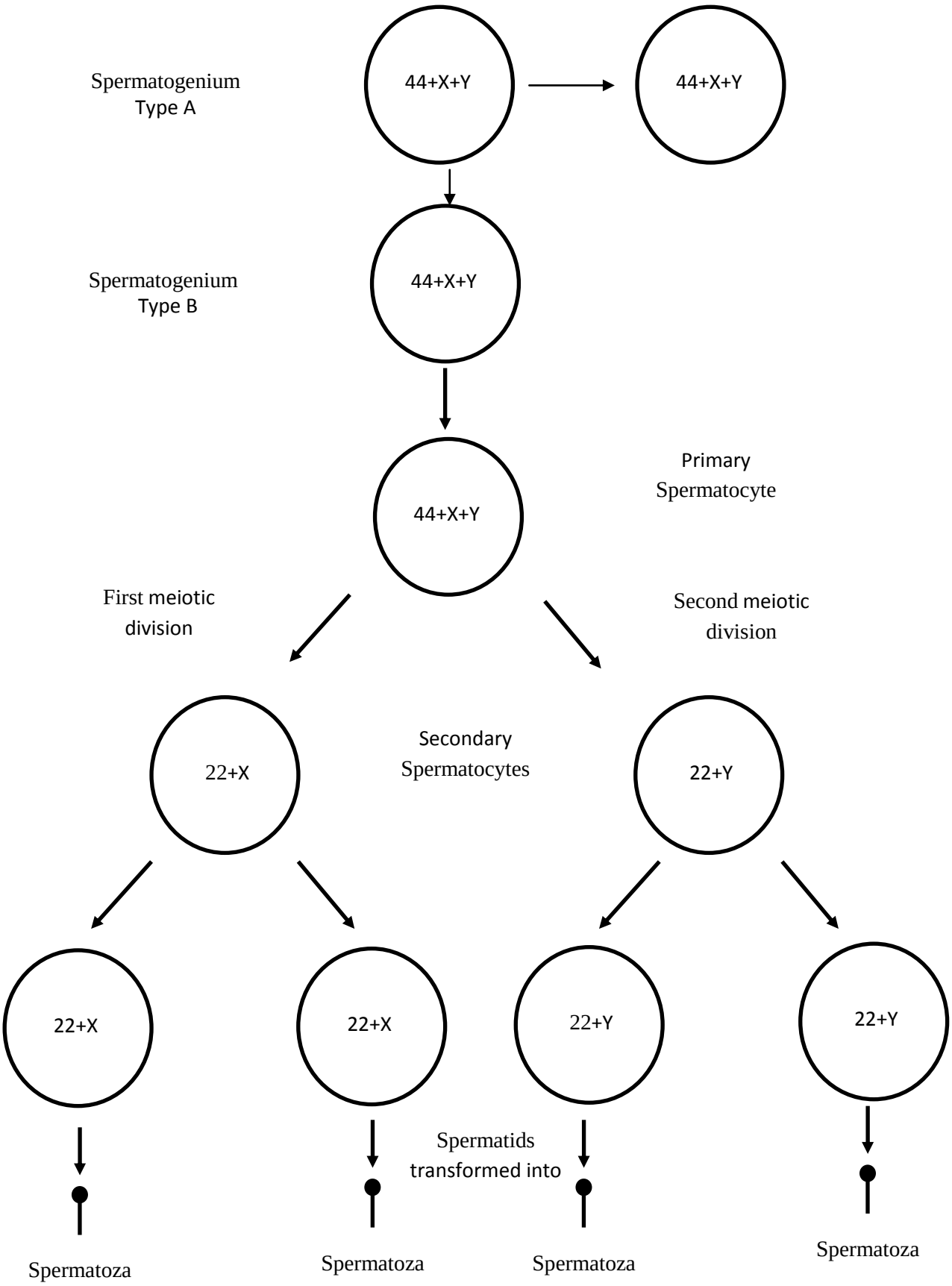
Stages in Spermatogenesis

Primordial germ cells
↓
Spermatogenesis
↓
Primary spermatocytes
↓
Secondary spermatocytes
↓
Spermatids
↓
Spermatozoa

Physiology of the Mature Sperm

The activity of sperm is greatly enhanced in a neutral and slightly alkaline medium as exists in the ejaculated semen but it is greatly depressed in a mildly acidic medium. A strong acidic medium can cause rapid death of sperm. The activity of sperm increases markedly with increasing temperature. Although the sperm can live for many weeks in the suppressed state in the genital ducts of the testes. Life expectancy of ejaculated sperm in the female genital tract is only 1 to 2 days.

Stages in Spermatogenesis



Maturation of sperm in the epididymis

After formation in the seminiferous tubules, the sperm require several days to pass through the 6-meter long tubule of the epididymis. Sperm removed from the seminiferous tubules and from the early portions of the epididymis are non-mortile, and they cannot fertilize an ovum. However after the sperm have been in epididymis for some 18 to 24 hours they develop the capability of motility until after ejaculation.

Storage of sperm

The two testes of the human adult form up to 120 million sperm each day. A small quantity of these can be stored in the epididymis but most are stored in the vas deferens. They can remain stored maintaining their fertility for at least a month. During this time they are kept in a deeply suppressed inactive state by multiple inhibitory substances in the secretions of the duct. Conversely with a high level of sexual activity and ejaculations storage may be no longer than a few days. After ejaculation the sperm become motile and they also become capable of fertilizing the ovum a process called maturation the sertoli cells and the epithelium of the epididymis secrete a special nutrient fluid that is along ejaculated with sperm. This fluid contains hormones and enzymes and special nutrients that are essential for sperm maturation.

Functions of seminal vesicle secretion[34]

Nutrition to Sperm

The fructose and other nutritive substances from seminal vesicles are utilized by sperm after being ejaculated into female genital tract.

Clotting of Semen

The fibrinogen from secretions of seminal vesicle is converted in to the coagulum as soon as semen is ejaculated.

On Fertilization

Causing reverse peristaltic movement of uterus and fallopian tubes. This, in turn, increases the rate of transport of sperms in female genital tract during coitus.

Table No.2. Secretary products of the cells in the testis

Sl.No	Cell Products	Proposed Function
A.	**Leydig Cells** 1. Androgens 2. Proopiomelanocortin 3. Inhibin 4. IGF – I 5. IL - 1β	1. Endocrine, paracrine, autocrine control 2. Opioids, α-MSH, ACTH 3. Endocrine, paracrine activity 4. Growth, differentiation 5. Kinin activity
B.	**Sertoli Cells** **1.** ABP **2.** Transferrin **3.** Ceruloplasmin **4.** Estrogen/Aromatase **5.** Laminin, Collagen **6.** Proteoglycans **7.** TFG-α, TFG-β, IGF-I, IL-I	1. Binding of androgens 2. Iron Transport 3. Copper Transport 4. Endocrine, paracrine regulation 5. Extracellular matrix 6. Growth factors which inhibit or stimulate cell physiology and proliferation
C.	**SC-EGF** 1. Inhibin, Activin 2. Mullerian, inhibitory factor 3. LHRH-Like substance 4. Lactate / Pyruvate	1. Endocrine, paracine 2. Fetal Sertoli cell development 3. Binds of leydig cells in rats 4. Metabolites, nutrients for gem cells.
D.	**Peritubular Cells** 1. P-Mod-S 2. Fibronectin 3. Proteoglycans 4. TFG-α, TFG-β, IGF-I	1. Paracine regulation od\f Sertoli cells 2. Extra cellular matrix 3. Growth factors

Stage of Spermatogenesis – Necessary Hormones[35A]

- Stage of proliferation - Growth Hormone, Testosterone
- Stage of growth - Testosterone, Growth Hormone
- Stage of maturation - Testosterone
- Stage of transformation - Testosterone, Estrogen and FSH

Hormonal factors that stimulate Spermatogenesis[35B]

Testosterone

It secreted by the leydig cells. Located in the interstitium of the testis, is essential for growth and division of the germinal cells in forming sperm.

Luteinizing Hormone

Secreted by the anterior pituitary gland, stimulates the leydig cells to secrete testosterone.

Follicle – Stimulating Hormone

FSH is also secreted by the anterior pituitary gland, stimulates the sertoli cells. Without this stimulation the conversion of the spermatids to sperm will not occur.

Estrogens

It formed from testosterone by the sertoli cells, when FSH stimulates them. It is probably also essential for spermatogenesis.

The sertoli cells also secrete an androgen-binding protein that binds both testosterone and estrogens and carries these into the fluid in the seminiferous tubular lumen thus making both these hormones available for maturing the sperm.

Growth Hormone

It is necessary for controlling the back ground metabolic functions of the testes. Growth hormone especially promotes early division of the spermatogonia themselves. In its absence as in pituitary dwarfs, spermatogenesis is severally deficient or absent.

Inhibin

Inhibin is a peptide belonging to transforming growth factor family. It is secreted by sertoli cells. It is also secreted in females by granulose cells of ovarian follicles. Its secretion is stimulated by FSH and inhibited by GnRH. Inhibin plays an important role in the regulation of spermatogenesis by inhibiting. FSH secretion through feedback mechanism.

Activin

Recently the peptide called activin is also found to be secreted in gonads along with inhibin. The exact location of it is secretion in testis is not known clearly. It is suggested that secreted by leydig cells and sertoli cells. Active has opposite actions of inhibin. It increases secretion of FSH and accelerates spermatogenesis.

The Hypothalamic – Pituitary – Testicular Axis

The hypothalamic – pituitary – testicular axis is physiologically a closely integrated system.

Testicular function is regulated by a series of closed-loop feedback systems involving the higher centre in the central nervous systems (CNS), the hypothalamus, the pituitary and the testicular, endocrine and germinal compartments.

The hypothalamus is the site of production of gonado-tropin-releasing hormone (GnRH). GnRH binds to GnRH receptors in the pituitary gland and stimulates the synthesis and release of the Gonadotropic Hormones, Luteinizing Hormone (LH) and Follicle stimulating Hormone (FSH).

LH and FSH are secreted by the pituitary gland into the general circulation and carried to the testes. In the testis they stimulate gonadal secretion of steroid hormones (testosterone and estradiol) that are important in the maturation and maintenance of spermatogenesis.

Testosterone is the major steroid hormone produced by the testis. 98% of testosterone circulates bound either to sex hormone – binding globulin (SHBG) or to albumin. The testis secretes only 25% of circulating estradiol. Dihydro testosterone (DHT) another potent androgen is derived from the peripheral conversion of

testosterone. This DHT is necessary for external virilization during embryogenesis and androgen action during puberty and adulthood.

The testis also produces non-steroid substance inhibin, secreted by the sertoli cells. Inhibin may also exert local regulatory effects on spermatogenesis.

Prolactin a polypeptide hormone is synthesized and secreted from the pituitary gland. Prolactin stimulates lactation in women. Elevated levels of prolactin suppress testosterone synthesis in man. Control and coordination of testicular function occur via feedback signals both positive and negative exerted by the hormones secreted at each level of the hypothalamic-pituitary-testicular axis.

Normal male sexual function requires coordinated regulation of the following physiologic events: libido or sexual desire, sustained penile tumescence or erection, ejaculation, orgasm, and detumescence[36].

Nutrition

Zin

Zinc is the most important nutrient mineral influencing male fertility. Zinc level in the seminal plasma is directly related to sperm motility. Dietary zinc restriction reduces both sperm count and seminal plasma volume. Zinc levels in seminal plasma of normal, oligospermic, asthenospermic and azoospermic subjects show that a linear direct relationship seems to exist between zinc in seminal plasma and motility of spermatozoa. Dietary restriction of zinc can affect testicular function adversely. This is the effect of zinc deficiency. The serum testosterone concentration and seminal volume are most sensitive to zinc depletion in men in the reproductive period.

Vitamin B12

Vitamin B12 deficiency also plays a role in fertility. “Intrinsic factor” is necessary for the proper absorption of B12 and its deficiency is one of the causes of secondary infertility in male.

Vitamin C

Studies have shown the concentration of ascorbic acid in seminal plasma directly reflects dietary intake, and lower levels of vitamin C may lead to infertility and increased damage to the sperm's genetic material.3 Fraga et. al demonstrated this by reducing ascorbic acid intake in healthy men from 250mg to 500mg per day. Seminal plasma levels of vitamin C decreased by 50 percent, with a concomitant 91-percent increase in sperm with DNA damage.

L-arginine

The biochemical and physiological relevance of L-arginine lies in its role as the precursor in the synthesis of polyamines and testosterone. The polyamines putrescence and spermidine are organic components important to sperm motility. Arginine metabolism is a factor in normal sperm production being involved as a source of nitric oxide within spermatozoa. Nitric oxide (at endogenous concentrations) appears to be necessary for adequate sperm motility. The endothelial (eNOS) and brain (bNOS) nitric oxide syntheses are abundant in normozoospermic samples but is low in asthenozoospermic patients. Consequently, an adequate dietary amount of L-arginine is necessary for normal spermatogenesis, especially for the sperm motility and arginine aspartate (9 g daily) has been found to be effective in some cases of asthenospermia. L-arginine, 4 gm daily has been shown to improve sperm counts in men with oligospermia. Nuts, oilsees, flesh foods, pulses and legumes are common sources of L-arginine.

Vitamin E

The membranes of the germ cells and spermatozoa are very sensitive to oxidation because of their high content of PUFA (Polyunsaturated fatty acids). Vitamin E is a major lipophilic chain-breaking anti oxidant, which protects tissue PUFA against peroxidation, a property that is beneficial in the male reproductive physiology. Oral administration of vitamin E significantly improves the in vitro function function of human spermatozoa as assessed by the zonabinding test. Vitamin E antioxidant therapy is however, dependent on the dosage or the in vitro concentration of the vitamin. Vitamin E in a dose of 200 IU twice daily acts an antioxidant and improves sperms ability to impregnate.

Selenium

Men with reduced sperm motility, supplementation with selenium (100 mcg per day for three months) significantly increased sperm motility, but it had no effect on sperm count. Selenium is one of the important ingredients that is very often lacking in order mean and can be found in horsetail, which has been used with success in ED following prostatic enlargement.

L-Carnitine

Sperm motility also increased both in quantitative and qualitative manners. In a multicentric study, increase in the sperm motility was also observed in terms of both rapid linear progression and linearity index along with that the sperm output after oral administration of L – Carnitine in patients with idiopathic asthenozoospermias. Two amino acids Iysine and methionine that is necessary for the biosynthesis of L-Carnitine in the body.

Antioxidants

Polyunsaturated fatty acids and phospholipids are key constituents is the sperm cell membrane and are highly susceptible to oxidative damage. Sperm produce controlled concentrations of reactive oxygen species, such as the superoxide anion, hydrogen peroxide, and nitric oxide, which are needed for fertilization; however, high concentration of these free radicals can directly damage sperm cells. Disruption of this delicate balance has been proposed as one of the possible etiologies of idiopathic male infertility. About some Anti-oxidants,

- Vitamin A alone improved sperm function and IVF rates in studies.
- Vitamin A, Vitamin E and essential fatty acids (omega-3 fats) were shown to increase sperm count in another study.
- Folic acid and zinc may increase sperm concentration.
- The bottom line: having a healthy diet is important for male fertility. If you are having trouble conceiving, re-evaluate your diet. You should have a diet rich in a variety fruits and vegetables and take a good quality multivitamin daily. You may also consider taking an omega-3 supplement, if your intake of fish is low.

Coenzyme Q-10

In sperm cells, coenzyme Q10 (CoQ10) is concentrated in the mitochondrial mid-piece, where it is involved in energy production. It also functions as an antioxidant, preventing lipid peroxidation of sperm membranes. When sperm samples from 22 asthenospermic men were incubated in vitro with 50 microM CoQ10, significant increases in motility were observed. CoQ10 (60 mg) was given to 17 infertile patients for a mean 103 days, and although there were no significant changes are ($p<0.05$).52 In another study, 10 mg/day of coenzymes Q7 (an analog of CoQ10) was given to infertile men, with resulting increases in sperm count and motility.

Infertility History

History of Infertility

- Duration
- Prior Pregnancies
- Previous treatments
- Evaluation and treatment of wife
- Present partner
- Another partner

Sexual History of the Man

- Frequency of masturbation
- Frequency of intercourse,
- Timing of intercourse
- Potency
- Lubricants

Childhood and Development

- Undescended testicles
- Herniorraphy
- Testicular trauma
- Testicular torsion
- Y-U plasty of bladder
- Onset of puberty-early, normal of delayed

Family History

- History of infertility in his family members
- Cystic fibrosis
- Androgen receptor deficiency

Infections

- Viral infections
- Febrile
- Sexually transmitted disease
- Tuberculosis
- Chicken pox
- small pox
- Mumps
- Orchitis

Surgical History

- Pelvic injury
- Orchitectomy
- Herniorraphy
- Pelvic injury or scrotal swelling
- Y-V plasty
- Retro peritoneal surgery

Gonadotoxins

- Thermal exposure
- radiation
- Smoking
- Chemicals – Pesticides
- Drugs, Chemotheraputic, Marijuana, Sulfasalazine, Nitro furantoin, Androgentic steroids.

Causes of Infertility

Varicocele

A varicocele is defined as a dilation of the veins of the pampiniform plexus of the scrotum. Varicocele is present in 15% of the male population. Dilated spermatic vein leads to the reflux of toxins (impure blood-increased CO_2 concentration produces excessive heat) down through the spermatic vein to the testis. That increases scrotal temperature caused by varicocele.

High Fever

A high fever exceeding 38^0C may suppress spermatogenesis over a period of 6 months. Example: Influenza, malaria.

Testicular trauma

Testicular trauma is the second most common acquired cause of infertility the testes are at risk for both thermal and physical trauma because of their exposed position.

Orchitis

The most common cause of acquired testicular failure in adults is viral orchitis usually caused by the mumps virus, echovirus or group B arbovirus.

Down syndrome

These patients have mild testicular dysfunction with varying degrees of reduction in germ cell number. LH and FSH are usually elevated.

Sertoli – cell only syndrome (Germinal cell aplasia)

Patients with germinal cell aplasia have LH and testosterone levels within the reference range but have an increased FSH level. The etiology is unknown but is probably multi-factorial patients present with small to normal sized testes and azoospermia.

Secondary sex characteristics are normal. Histology reveals seminiferous tubules lined by sertoli cells and a normal interstitum although no germ cells are present.

Commoner Abnormalities of Genital Organs

- Local infection
- Idiopathic
- Testicular trauma of Torsion
- Varicocele
- Obstruction of epididymis,
- Obstruction of vas deferens
- Cryptorchidism

Chronic Diseases

- Mumps
- Tuberculosis
- Leprosy
- Epididymitis
- Prostatitis
- Diabetes mellitus
- Hypertension
- Sexual transmitted diseases

Testicular Changes in Infertility

The causes of infertility are classified as pretesticular, testicular and posttesticular.

Pretesticular Causes

- Hypothalamic disease- Isolated gonadotrophin deficiency (Kallmann's syndrome)
- Isolated LH deficiency (Fertile eunuch)
- Isolated FSH deficiency
- Congenital hypogonadrotrophic syndrome
- Haemochromatosis

- Exogenous hormones (estrogen-androgen excess)
- Glucocorticoid excess
- Hyper and hypothyroidism
- Drugs, alcohol, smoking
- Strenuous riding (bicycle riding, horseback riding)

Testicular Factors

- Genetic defects on the Y chromosome
- Abnormal set of chromosomes (klinefelter syndrome)
- Neoplasm (seminoma)
- Cryptorchidism
- Varicocle
- Trauma
- Hydrocele
- Mumps
- Malaria
- Testicular dysgenesis syndrome

Post Testicular Causes of Infertility

- Vas deferens obstruction
- Lack of Vas deferens, often related to genetic markers for Cystic Fibrosis
- Infection, e.g. prostatitis
- Retrograde ejaculation
- Hypospadias
- Impotence
- Acrosomal defect/egg penetration defect

Azoospermia or severe oligospermia in a patient whose testicular biopsy findings are normal is indicative of obstructive, post testicular infertility. The site of obstruction is less determined during surgical exploration or by a vasogram.

On the basis of testicular morphology, primary disturbances of spermatogenesis can be subdivided into three groups, germ cell aplasia, maturation arrest of spermatogenesis and hypo spermatogenesis.

Germ cell aplasia, or sertoli syndrome, is the secere of these disturbances and is invariably accompanied azoospermia.

Maturation arrest of Spermatogenesis is characterized by incomplete spermatogenesis. The maturation arrest can be at any stage of spermatogenesis but most often occurs at the level of the primary spermatocytes the term incomplete maturation arrest is used to refer those cases in which the tubules show arrest at different stages of spermatogenesis. In such cases, some tubules show spermatogenic cells arrested at one stage (for example, spermatids and adjacent tubules arrested at another stage (for example, primary spermatocytes). Hypospermatogenesis is a quantitative reduction in the number of spermatozoa produced by spermatogenesis.

Erectile dysfunction

Erectile dysfunction is also known as impotence, is the inability to achieve or sustain an erection for satisfactory sexual activity. This is differ from other conditions that interfere with male sexual intercourse, such as lack of sexual desireness (decrease libido), and problems with ejaculation and orgasm (ejaculatory dysfunction).

Approximately 35 % of men 40-70 years of age suffer from moderate to severe erectile dysfunction. 50 % of men over the age of 40, having erectile dysfunction, 10 % of men below 40.

Physiology of erection

- An erection begins in the brain, physical or mental stimulation cause nerves in the brain to send chemical messages to nerves in the penis telling the blood vessels to relax so that can flow freely in to the penis.
- Once in the penis, high pressure traps the blood with in both corpora cavernosa. This causes the penis to expand and sustain an erection.
- Erection is reversed when the inflow of blood is stopped and opening outflow channel open, allowing the penis to become soft.

-Human physiology, Guyton

Molcular mechanism of erection

- During sexual excitement, nitric oxide is released from the cavernous nerve (which control erection), nitric oxide activates an enzyme in smooth muscle called guanylyl cyclase.

- This activated enzyme in turn transforms GTP, an important energy source inside cells, in to cGMP.
- cGMP is a molecule that through a complex process causes smooth muscle relaxation,leading to dilatation of arteries and the rapid filling of the spongy erectile tissues.

Nerve supply

- Parasympathetic supply- vasomotor supply $S_{2,3,4}$ (Nervi ergentes)
  It causes dilatation of helicine arteries during the erection of penis.
- Sympathetic supply - Vaso constrictor nerves of the penile vasculature.
  Sympathetic from L_1 segment reaches the penis through the branches of pudendal nerve.
- Somatic sensation -carried out by the dorsal nerve of the penis, they supply the skin and muscle acting on penis.

Neuro transmitters

- The erectile mechanism is mainly driven by the acetyl choline-parasympathetic action, which produce the nitric oxide (erectile neurotransmitter) and c GMP (erectile dilator).
- On the other hand, the dopamine and nor epinephrine axis boosts libido and promotes the burning of testosterone, which expands the erectile tissues to an extreme state.
- Then, a large amount of nor-epinephrine becomes epinephrine (adrenaline) by conversion.
- That triggers the sympathetic 'Fight or Flight' command, where Fight means ejaculation and orgasm (if acetyl choline action is sufficient) and Flight the erection or engorgement withdrawal (if acetyl choline action is insufficient).
- Serotonin can block or reduce nor epinephrine-adrenaline conversion (adrenaline is the main enemy of erection or engorgement)
- In the foreplay and erectile states the stress hormone (adrenaline) can also block the incoming parasympathetic motoring communication from the brain to the targeted organ.

- Without stress hormone and with a high testosterone level, the parasympathetic nervous communications from the brain to the sex organs- the erection circuit will be frequently turned on.

Male sexual response cycle

Based on the literature support the normal male sexual response cycle can be functionally divided into five interrelated events that occur in a defined sequence: libido, erection, ejaculation, orgasm, and detumescence.

❖ **Libido or sexual desire**

Libido is defined as the biological need for sexual activity (the sex drive) and frequently is expressed as sex-seeking behaviour. Its intensity is variable between individuals as well as within an individual over a given time. Higher serum testosterone appears to be associated with greater sexual activity in healthy older but not younger men.

❖ **Erection**

Erection is the enlarged and rigid state of the sexually aroused penis sufficient enough for vaginal penetration. It results from multiple psychogenic and sensory stimuli arising from imaginative, visual, auditory, olfactory, gustatory, tactile, and genital reflexogenic sources.

❖ **Ejaculation**

Ejaculation is the act of ejecting semen. It is a reflex action that occurs as a result of sexual stimulation. It is made up of two sequential processes. The first process called emission is associated with deposition of seminal fluid into the posterior urethra while the second process is the true ejaculation, which is the expulsion of the seminal fluid from the posterior urethra through the penile meatus.

❖ **Orgasm**

This is the climax of sexual excitement. The entire period of emission and ejaculation is known as the male orgasm.

❖ **Detumescence**

This is the subsidence of an erect penis after ejaculation.

Male sexual dysfunction-Impotence

Sex disorders of the male are classified into disorders of sexual function, sexual orientation, and sexual behaviour. In general, several factors must work in harmony to maintain normal sexual function. Such factors include neural activity, vascular events, intracavernosal nitric oxide system and androgens. Thus, malfunctioning of at least one of these could lead to sexual dysfunction of any kind. Sexual dysfunction in men refers to repeated inability to achieve normal sexual intercourse. It can also be viewed as disorders that interfere with a full sexual response cycle. These disorders make it difficult for a person to enjoy or to have sexual intercourse. While sexual dysfunction rarely threatens physical health, it can take a heavy psychological toll, bringing on depression, anxiety, and debilitating feelings of inadequacy.

Unfortunately, it is a problem often neglected by the health care team who strive more with the technical and more medically manageable aspects of the patient's illness. Sexual dysfunction is more prevalent in males than in females and thus, it is conventional to focus more on male sexual difficulties. It has been discovered that men between 17 and 96 years old could suffer sexual dysfunction as a result of psychological or physical health problems. Generally, a prevalence of about 10% occurs across all ages. Because sexual dysfunction is an inevitable process of aging, the prevalence is over 50% in men between 50 and 70 years of age. As men age, the absolute number of Leydig cells decreases by about 40%, and the vigour of pulsatile lutenizing hormone release is dampened. In association with these events, free testosterone level also declines by approximately 1.2% per year. These have contributed in no small measure to prevalence of sexual dysfunction in the aged.

Sexual dysfunction takes different forms in men. A dysfunction can be life-long and always present, acquired, situational, or generalized, occurring despite the situation. Male sexual dysfunction can be categorized as disorders of desire, disorders of orgasm, erectile dysfunction, disorder of ejaculation and failure of detumescence.

A. Disorders of desire

Disorders of desire can involve either a deficient or compulsive desire for sexual activity. Dysfunctions that can occur during the desire phase include:

(i). Hypoactive sexual desire

Defined as persistently or recurrently deficient (or absent) sexual fantasy and desire for sexual activity leading to marked distress or interpersonal difficulty. It results in a complete or almost complete lack of desire to have any type of sexual relation.

(ii). Compulsive sexual behaviours

Constitute a wide range of complex sexual behaviours that have strikingly repetitive, compelling, or driven qualities. They usually manifest as obsessive-compulsive sexuality (e.g. excessive masturbation and promiscuity), excessive sex-seeking in association with affective disorders (e.g. major depression or mood disorders), addictive sexuality (e.g. attachment to another person, object, or sensation for sexual gratification to the exclusion of everything else), and sexual impulsivity (failure to resist an impulse or temptation for sexual behaviour that is harmful to self or others such as exhibitionism, rape, or child molestation).

B. Erectile dysfunction (ED)

This is a problem with sexual arousal. ED can be defined as the difficulty in achieving or maintaining an erection sufficient for sexual activity or penetration, at least 50% of the time, for a period of six months. It results in significant psychological, social and physical morbidity, and annihilates his essence of masculinity.

C. Disorders of ejaculation

There exists a spectrum of disorders of ejaculation ranging from mild premature to severely retard or absent ejaculation. These include:

(i).Premature ejaculation: Which is the most common male sexual dysfunction and can be any of the following: a) persistent or recurrent ejaculation with minimum sexual stimulation that occurs before, upon, or shortly after penetration and before the person wishes it; b) marked distress or interpersonal difficulty; and c) the condition does not arise as a direct effect of substance abuse. Premature ejaculation and sexual desire disorders were the frequent reported problems in young adult males with adverse familial relationship**.**

(ii).Painful ejaculation*:* which results from side effect of tricyclic antidepressants is a persistent and recurrent pain in the genital organs during ejaculation or immediately afterwards**.**

(iii).Inhibited or retarded ejaculation: This is when ejaculation does not occur at all.

(iv).Retrograde ejaculation: This is when ejaculation is forced back into the bladder rather than through the urethra and out of the end of the penis at orgasm.

D. Disorders of orgasm

Male orgasmic disorder is defined as a persistent or recurrent delaying or absence of orgasm after a normal sexual excitement phase during sexual activity.

E. Failure of detumescence

It is a prolonged erection usually lasting for between 4 h or greater. It is painful and always unaccompanied by sexual desire despite the fact that it is often preceded by usual sexual stimuli. Diagnostic options for male sexual dysfunction include: patient's history which embodies medical history (evaluating historical events like chronic disease, pharmacological agents, endocrine disorders, surgeries and trauma), psychological history (assessing individual's upbringing relationships, early sexual experiences, inadequate sexual information and general psychological health), sexual history (to ascertain the time and manner of onset, its course, current status, and associated medical or psychological problems), physical examination (entails general and systemic evaluation, assessment of gonadal function, vascular competence, neurological integrity, and genital organ normalcy), diagnosis testing (include blood tests, vascular assessment, sensory testing and nocturnal penile tumescence and rigidity testing).

Types of erectile dysfunction

1. Arteriogenic
2. Neurogenic
3. Endocrinologic
4. Mixed
5. Psychogenic

Primary- Impotence since birth

Secondary- Impotence sets in after years of normal sex

Causes

Psychological causes

These factors are responsible for about 10%-20% of all cases.

It is often a secondary reaction to an underlying physical cause.

- Stress
- performance Anxiety
- Guilt
- Depression
- Low self esteem
- Fear of sexual failure

Physical causes

- Aging (decrease In hormonal level with age).
- Chronic medical conditions (diabetes, hypertension).
- Vascular insufficiency (atherosclerosis, venous leakage).
- Penile disease (Peyronie's, priapism, phinosis, smooth muscle dysfunction)
- Pelvic surgery (to correct arterial or inflow disorder).
- Neurological disorders (Parkinson's disease, stroke, cerebral trauma, Alzhemier's spinal cord or nerve injury).
- Drugs (side effects -anti-hypertensives, central agents, psychiatric medications, antiulcer, antidepressants, and anti-androgens.)
- Systemic diseases (cardiac, hepatic, renal, pulmonary, cancer, metabolic, post-organ transplant).
- Life style (chronic alcohol abuse, cigarette smoking).
- Androgen deficiencies (testosterone deficiency, hyperprolactinemia).

Chronic anxiety and sexual activity

- It is an indicator that there are modulation disorders of serotonin and GABA on the autonomic nervous system.It usually leads to overpowered nervous function this in turn leads to premature ejaculation and performance anxiety.

- Generally the serotonin and GABA modulate the nor adrenergic-sympathetic nervous function in two days.
- By modulating the ANS-Autonomic nervous system via direct nor epinephrine neurons of the hypothalamic locus coeruleus (LC).
- By modulating the neuro endocrine humoral outflow via the hypothalamic-pituitary-adrenal axis.
- The serotonin, GABA and endorphin nervous control can reduce seminal vesicles, prostate and penile nervous sensitivity.
- Also the GABA-erginic nerves control the non adrenergic firing in the LC while the serotonin-ergic nerves reduce the non adrenergic sympathetic nervous function.
- Sexual stimulation or orgasm ignites conversion of dopamine-nor epinephrine in the hypothalamus and adrenal medulla, after that nor-epinephrine neurons of the locus coeruleus are activated. ProstoglandinE_2 is stimulated by the nor epinephrine.
- Prostaglandin E_2 sensitizes the sympathetic nerves in the adrenal glands, seminal vesicles, testicles, penis and prostate for the nor epinephrine release.
- Nor epinephrine release, in turn, triggers the neurotransmitters prostaglandin E_1 and prostaglandin E_2 release.
- The release of Prostaglandins triggers the nervous erectile mechanism (with the release of Nitric oxide and cGMP) that leads to erection.
- However, high levels of Prostoglandin E_2 may over sensitize the sympathetic and somatic nerves located in the prostate, bulbo urethral glands and penis, which in turn inflame the organ and will cause numerous symptoms, such as fast ejaculation(even within seconds) and precum leakage.

Current treatment strategy

The first anti-impotence drug, alprostadil, was marketed in 1995 and is available as a local injection or an intraurethral pellet. The latter method of delivery was discovered after it was noted that drugs could be absorbed into the cavernosal bodies through the walls of the urethra. More recently oral therapies for the treatment of impotence have been marketed, starting with the accidental discovery of sildenafil citrate (Viagra).

In 1991, researchers discovered that chemical compounds belonging to the pyrazolopyrimidinone class were useful for treating cardiac conditions such as angina. In 1994, whilst trials for this were underway (with little success) it was noted that the drug also increased blood flow to the penis and therefore increased erections. Subsequently, in 1998, the FDA gave approval for sildenafil citrate as the first oral anti-impotence drug. With the latest addition, apomorphine (Uprima), there is now a race to produce a pill with a faster onset of erection, fewer side effects, available to all patients and with no restriction on the frequency of use.

- Making lifestyle improvements (for example quitting smoking and exercise more)
- Taking drugs (PDE5 inhibitors)
- Intra urethral suppositories
- Intra cavernosal injections
- Vaccum constrictive device
- Penile prostheses
- Psychotherapy

Impaired Sperm Production and Function

1. **Hypothalamic pituitary disorders**

Congenital hypo-gonadotropic hypogonadism and kallmann syndrome due to deficiency of gonadotrophin releasing hormone (GnRH). In the X-linked form of this disease, deletion of a gene kalig-2 has been found. This gene encodes for neurons involved in production of GnRH. Acquired hypogonadotrophic hypogonadism pituitary adenoma (including prolactinomas) craniopharyngiomas other brain tumours, intracranial radiation therapy.

2. **Genetic Factors**

Sex chromosome abnormalities

a) 47-xxy karyotype (Klinefelter syndrome) is almost always associated with azoospermia (destruction of seminiferous tubules at puberty leading to shrinkage of testes).
b) Extra Y chromosome results in various degrees of impairment of spermatogenesis. Characteristic decreased sexual function, gynaecomazia, decreased length of penis and testis and decreased testosterone level.

Other chromosomal abnormalities

a) Translocations cause more severe impairment in male than female.
b) Impaired chromosome pairing in meiosis leads to azoospermia.

Deletions corresponding to AZF (Human azoospermia factor) region on long arm of chromosome. This genetic region controls spermatogenesis in human beings.

3. Undescended Tetsis (Cryptorchidism)

Extent of impairment of spermatogenesis is variable from a complete sertoli-cell only pattern to only a slight reduction in the number of germ cells. Spermatogenesis is also impaired in contra lateral testis in patients with unilateral mal-descent. Early treatment before age of two years is advocated.

4. Testicular Cancer

This is associated with increased risk of impaired spermatogenesis oligospermia is observed in more than 40% of patients at the time of diagnosis of testicular cancer.

5. Germ cell Aplasia

Seminiferous tubules contain only sertoli cells. Absence of germ cells may be due to factor present during fetal life. Leydig cell insuffiency may also be associated.

This cytological appearance can also result from cryptorchidism, cytotoxic drugs or irradiation.

6. Drugs

Sulphasalazine, used to treat inflammatory bowel disease can markedly reduce semen quality. The effect is reversible if smaller doses are used for limited time else it may be permanent, B-Blockers may cause importance Anabolic steroids may cause oligo (or) azoospermia and cytotoxic drugs especially the alkylating agents (Cyclophosphamide, cisplatin and procarbazine) also cause gonadal failure.

7. Environmental Factors

Exogenous heat can impair spermatogenesis, Pesticides (Chlorinated nematocide dibromochloropropane – DBCP, Chlordane, carboxyl and

ethylenedibromide) glycol ethers (used in paintings, painting and adhesives) and metals (lead, calcium and mercury) have adverse effect on sperm production.

Male metal welders may be at increased risk of sub-fecundity. Several other environmental toxins can also have an adverse effect on male reproductive organs. Various hormonal metabolic and neural signals like stress, under nutrition, emotional upset and drugs can affect hypothalamic-GnRH pulse generator and thus, spermatogenesis.

Impaired sperm transport

1. **Autoimmune Infertility**

Spermatozoal antigens are shielded inside the testes and are not recognized by the immune system. Autoimmune reaction against sperms is manifested as circulating sperm antibodies. These antibodies may be associated with vasectomy, unilateral or bilateral obstruction of genital tract, Epididymis and varicocele.

2. **Obstructive Azoospermia**

Obstructive azoospermia is a common cause of male infertility and can result from infection, congenital anomalies, or iatrogenic injury.

3. **Sexually transmitted diseases**

Sexually transmitted diseases may cause epididymitis and block the ductal system. Agenesis of epididymis and other parts of ductal system, congenital bilateral agenesis of vas deferens is found in many patients with cystic fibrosis.

3. **An Ejaculation / Retrograde Ejaculation**

- Diabetic patients
- Retroperitoneal lymph-node dissection causing neural damage.
- Spinal card injury
- Bladder neck surgery
- Other sexual dysfunction-including impotence
- Kartagener's syndrome (immotile ciliary syndrome) sperms are immotile due to missing dynein arms.

4. Disturbance in sperm oocyte function

Complementary adhesion molecules are present on surface of oocytes and spermatozoa. These molecules interact and cause fusion of gametes. Abnormalities in these molecules may potentially contribute to infertility.

5. Unexplained Infertility

Semen quality is normal and no effect can be found in the female partner.

Evaluation of the infertile man

I. This includes,
   - A comprehensive history,
   - Physical examination
   - Multiple semen analysis and
   - Endocrine evaluation

II. In special circumstances, further specific investigations may also be indicated.
   - Bacterial examination
   - Genetic assessment
   - Testicular biopsy
   - Sperm function tests
   - Ultrasound

A) History

Sexual history

About 5 percent of all couples are barren due to sexual dysfunction.

Smoking

Reduces sperm density, reduces the proportion of motile sperms and increase level of abnormal morphology.

Alcohol

Excess intake impairs liver function leading to increased estrogen levels, decreased sexual performance and depressed spermatogenesis.

Drug abuse

Marijuana can lead to impotence and infertility marijuana inhibits the secretion of GnRH and can suppress reproductive functions in both men and women and cocaine use is known to reduce – spermatogenesis.

Medical treatment

Psychiatric drugs like phenothiazines, anti-hypertensives like B-blockers, epileptic drugs like diphenyl hydantoin, anti-bacterials like Sulphasalazine and nitrofurantion, H_2 antagonist's cimetidine and ranitidine, erythromycin tetracycilne's anabolic steroids and chemotherapeutic agents can depress sperm quantity and quality.

Past history

Past history of mumps, orchitis, epididymitis, and prostatitis, sexual transmitted disease and testicular injury.

Exposure to excessive heat

A small rise in scrotal temperature can adversely affect spermatogenesis and a febrile illness can produce striking changes in sperm court and motility. The effect of the illness can be seen in the sperm count and motility even 2-3 months later. Environmental sources of heat such as the use of khaki shorts instead of boxes shorts excessively hot baths hot tubes or occupation that require long hours of sitting e.g. Drivers may all decrease fertility potential. Severe allergic reactions and exposure to radiation or to industrial or environmental toxins.

A study from Scandinavia did show lower sperm courts in males from an urban area compared to males in rural areas, suggesting an affect of urban pollutants. In any case the clinician should determine it a male with an abnormal semen specimen had exposure to industrial or environmental.

Exposure to diethyl stillbestrol in utero has been suggested (but not proven) as a cause of male infertility. Indeed in the largest follow-up of men born to the women treated with diethylstilbestrol, no impairment of fertility of sexual function was detected.

Coital frequency

Counts at the lower levels of the normal range may be depressed to below normal levels by ejaculations occurring daily or more frequently. Most couple's every 36 hours around the time of ovulation will give the optimal chance for pregnancy. However studies in men with Oligospermia fail to detect a decline in sperm-numbers with sequential ejaculations suggesting that in limitations on coital frequency are not necessary.

B. Examination

Bodily habitus

- Size of testes estimated by comparing with Orchidometer normal volume is – 150-200ml.
- Scrotal palpation for vas deferens and varicocele.
- Presence of penis abnormalities like hypospadias, scar and induration.
- Eunuchoid habitus with infantile genitalia, sparse body hair, gynecomastia and low testicular volume are seen in congenital gonadotrophin deficiency.
- Androgenized man with normal sized testis and distended epididymis may indicate obstructive azoospermia.
- Androgenized man small sized testes may have seminiferous tubular failure.
- Absence of cord like feel of vas of the neck of the scrotum indicates vasal aplasia.

Endocrine Evaluation

Serum follicle-stimulating hormone (FSH) helps to distinguish patients with azoospermia who have obstruction (normal FSH) from those with seminiferous tubule destruction(Raised FSH),Low levels of FSH, LH and testosterone suggest acquired hypo gonadotrophin hypogonadism.

C. Semen Analysis

The Semen analysis is the corner stone of the male infertility work up should be performed according to the WHO recommended procedure. Computer assisted semen analysis (CASA) is considered mainly a research tool and is not used

routinely. If abnormal results are obtained semen analysis is repeated after 6-12 weeks.

Semen volume **:** Normal ejaculate volume is 1.5-5ml.

Colour **:** Grey, yellow or opalescent

Ph **:** 7.2 – 8

Liquefaction **:** Coagulation occurs soon after ejaculation but semen liquefies within 5-20 minutes failure to liquefy after 30 min is abnormal.

Sperm concentration **:** Sperm per ml of semen

Normal - 20 x 10^6 sperms/ml

Polyzoospermia - 350 x 10^6/min

Total sperm count **:** Sperm concentration x volume of semen Normal)

40 x 10^6 sperms ejaculate.

Sperm Motility **:** At least 100 sperms are evaluated. Normal is 50% with forward progression within 60min of ejaculation.

Morphology **:** Assessed by light microscopy (hematoxylin, eosin, geimsa or papanicoloau stain) or electron microscopy. At least 100 sperms are examined. Normal is 30% with normal forms.

White blood cells **:** Have to be differentiated from immature germ cells. Normal (1 x 10^6/ ml peroxidase staining technique). If excessive, semen culture should be performed.

Sperm antibodies **:**Detected by immunobead or mixed antiglobulin reaction which localizes IgG or IgA specific regions of spermatozoa. Normal for immunobead test for antiglobulin reaction test – 10% spermatozoa with adherent particle.

Accessory gland functions **:** Assessed by measuring seminal fructose for seminal vesicles (Normal 13µmol/ejaculate) and acid phosphatase, zinc citrate for prostate. Post ejaculatory urine should be examined for presence of sperms if retrograde ejaculation is suspected.

Collection of semen

- The sample should be obtained atleast in three occasions.
- With an interval of atleast two months of each specimen.
- Atleast 4 day's abstinence from sexual activity.
- A sample to be collected by masturbation into a clean, dry, sterile container.
- The specimen (semen) should be examined should not be missed since it contains the highest concentration of spermatozoa.

Other Investigations

Bacteriological examination

Bacterial examination of semen in patients with leukocytospermia.

Chromosomal and genetic assessment

Chromosomal analysis is essential in men with azoospermia with raised FSH levels and small testicular volume, in order to diagnose klinefelter syndrome. Screening for cystic fibrosis-by-cystic fibrosis transmembrane conductance regulating (CFTR) gene analysis is done in men with congenital absence of vas deferens.

Testicular biopsy

Can confirm the diagnosis of obstructive azoospermia (Normal testis size with azoospermia) before reconstructive surgery.

Sperm function

- Strict morphology evaluation
- Acrosomal assessment
- Sperm – zone binding tests
- Production of reactive oxygen species by sperms.

Ultrasound

Transrectal and scrotal ultrasound can be used to assess prostate and seminal vesicle and diagnose ejaculatory duct obstruction. Testicular ultrasound can locate impalpable testes or those with hydrocele.

Other Parameters

Although all of the major elements of the semen analysis have some bearing on fertility, especially when markedly deficient the lack of precise correlations have led to a search for tests of the functional capacity of sperm. Despite enthusiasms generated by a variety of assays over the past four decades, no test has emerged as a reliable standard for the fertilizing ability of sperm.

Measurement of sperm velocity

The CASA systems are best at supplying information on sperm velocity and specific movements such as lateral head displacement however; it is unlikely that these measurements provide information that cannot be obtained with less expensive methods.

Hypo-osmotic swelling test

When sperm are placed in a hypo-osmotic solution of sodium citrate and fructose, a sperm tail with normal membrane function will swell and coil as fluid is transported across the membrane. Conversely, if there is a functional disturbance of the tail membrane the tail will appear unaffected.

Measurement of acrosin

Acrosin is a proteolytic enzyme associated with the acrosome that may be important for aiding sperm to traverse the zona.

Low acrosin concentrations could be associated with infertility. Although theoretically appealing, the test has little application in clinical practice.

Measurement of the acrosome reaction

The acrosome reaction that allows the release of enzymes from the acrosome occurs on or near the zona pellucida. However a low percentage of sperm will also become reactive while in media or following treatment with calcium, ionophores that includes capacitation. Although the artificial initiation of the acrosome reaction has been correlated with IVF results the relatively small difference in an acrosome reactive sperm in the different groups indicates that this approach is not clinically important.

Sperm Clumping or Agglutination

The sperms may clump head-to-head, tail-to-tail, or head-to-tail. In particular tail-to-tail agglutination of motile sperm is noteworthy and usually is followed up with Sperm Function.

Semen Culture Test

In a semen culture test, the semen sample is tested for the presence of bacteria. Testing the bacterial sensitivity to antibiotics is mandatory if there is any presence of bacteria. Whether the bacteria present in the specimen are that are usually seen in normal semen or those of a bacterial disease, without the evidence of inflammation or infection, there is no indication there is no indication for routine culture or antibiotic treatment in infertile men. If urine analysis is abnormal or bacterial prostatitis is suspected from the history or the physical examination, semen culture is certainly indicated. The common sexually transmitted organism such as Chlamydia trachomatis, Mycoplasms hominus and Ureaplasma urealyticulum have been implicated in reproductive failure in animals and humans.

Biochemical Test

Biochemical analysis of seminal plasma mostly provides insights into the function of the accessory sex glands. The fructose content of semen (normal value 250-400 mg or 4-28 mol/litre) should be routinely tested. Low fructose content (less than 120 mg) is often due to seminal vesiculitis, androgen deficiency or partial ejaculatory duct obstruction. Its absence indicates complete obstruction either due to a congenital block at the level of ejaculatory duct or proximal to it like agenesis of the vas and the seminal vesicle or following acquired post-infective cicatrisation. Almost invariably, these conditions are associated with azoospermia or severe oligospermia.The epididymis is represented by glyceryphosphorylcholine (GPC), the seminal vesicles by fructose and the prostate gland by zinc.

Immunological Test

In the enzyme-linked immune sorbent assay (ELISA) test, the antisperm antibodies measuring up to 20 units/min in 32 or 64 dilutions is considered normal.

Sperm Function Test

The sperm function tests assess the sperm's ability to fertilize the ovum. There is a drawback that these tests are often not standardized adequately.

Sperm Viability or Sperm Survival Test

The sperm viability may be determined by two methods-Eosin Y stain exclusion and hypo-osmotic swelling or HOS assay.

Bovine Cervical Mucus Test

The bovine cervical mucus test is another form of testing for the ability of the sperms to penetrate and swim through cervical mucus. These tests to assess the fertilizing potential of sperms. This in vitro functional test measures the ability of penetration of the sperms. The end point of this assay is penetration of the ovum and decondensation of sperm heads. Men with sperm of low SPA score are less likely to achieve a spontaneous pregnancy than those with high SPA score.

Sperm Chromatin Structural Assay (SCSA)

SCSA is a flow cytometric test where sperm DNA breaks can be evaluated indirectly through the DNA denaturability. The assay measures the susceptibility of sperm DNA to acid-induced DNA denaturation, followed by staining with the fluorescent dye acridine orange[37-39]. By using a flow cytometer, 5000–10 000 sperms can be analyzed within few seconds and thus provides a less subjective result compared with the WHO analysis where only 1–300 cells are analyzed. Through a specific SCSA software (SCSA-Soft; SCSA Diagnostics, Inc., Brookings, SD, USA), a scatter plot is created, showing the ratio of green and red sperm. The percentage of red sperm is called DNA fragmentation index (DFI)[40]. Visualized by a histogram. These are the sperms with denaturated DNA.

Sperm penetration assay (SPA)

The zona pellucida of most mammalian species presents not only a block to polyspermia but also a barrier to fertilization of an ovum by sperm of a different species. However, foreign sperm can fuse with and penetrate an ovum if the zona is removed by gentle enzyme digestion. In the sperm penetration assay, ovum are

collected from super ovulated golden hamsters the zona are removed by enzyme digestion, and the denuded ovum are cultured for 2-3 hours with human sperm that have been washed and incubated over night in culture media. Presence of a swollen sperm head in the ovum cytoplasm is evidence of successful penetration. Most laboratories report the percentage of ovum penetrated and compare this figure to the percent penetrated by a known fertile sperm specimen.

Human zona binding assay

Whereas the SPA tests the ability of sperm to penetrate or to be engulfed by the ovum it does not test the critical ability to pass through the zona pellucida.

The zona is of course, removed in preparation for the SPA because it is, with rare exceptions, impervious to foreign sperm. Thus, to test zona penetrating or zona binding ability of human sperm requires the use of human zona.

One approach is to use zona obtained from surgically removed ovarian tissue and slit them in half so that both patient sperm and donor sperm can be tested in parallel on different portions of the same zona.

The ratio of the number of sperm bound for the test subject to the number of sperm bound for fertile control sperm bound for fertile control sperm has been labeled the hemizona assay index (HZI) a break point at on HZI value of 36 has provided a good correlation with results in human IVF.

The limited availability of the zona and the technical requirements of the assay will always restrict its application to a small number of committed laboratories.

In the future, development of materials that mimic the properties of the zona could lead to simple tests.

However the widespread use of ICSI, which by passes the zona, renders such, tests superfluous, unless they can determine with certainty which couples require ICSI.

Measurement of the adenosine triphosphate (ATP)

ATP is an important component of sperm metabolism related to tail movement. In one reports levels of ATP in semen were a strong discriminator

between populations of fertile and infertile males. A multicentre study sponsored by the world health organization concluded. However, those levels of semen ATP could not predict the occurrence of pregnancy when the female was normal and the male partner had a sperm concentration greater than 20million/ml.

Immunological factors: Anti sperm antibodies

Immunological factors have been impacted in the causation of human infertility. In men, this may present as anti-sperm antibodies in the semen, serum or on the surface of the sperm. Anti-sperm antibodies have also been demonstrated in the cervical mucus and the serum of the female partner.

Though there are several tests to detect anti-sperm antibodies the exact significance of these tests is not known. Besides there is no satisfactory way to treat these couples, though immunosuppressive therapy with corticosteroids, testosterone therapy, intrauterine artificial insemination with husbands sperms (IUAID) and have all been suggested as appropriate therapy. The pregnancy rate has been variable.

The role of anti-sperm antibody is significant since the sperm is not recognized, as self-antigen in our body immunologically speaking the spermatogenesis does not occur, when the ontogeny of the 'T' lymphocytes happens. At this time of ontogeny of the 'T' lymphocytes our body proteins are started to recognize as self-antigens. It any antibody arises against our own protein it will be destroyed by clonal energy. The sequestered sperm protein also causes the immune response, especially vasectomy like surgeries.

Advanced sperm fertility tests

Computer-assisted semen analysis (CASA)

The new technologies such as CASA incorporate the video systems to measure the types and the speed of sperm motility, Normal sperm swim faster and straighter than the abnormal ones. The average speed of a human sperm is roughly 48 to 96 mm per second. CASA permits the measurement of additional motility parameters such a curvilinear velocity (VCL), straight-line velocity (VSL). Linearity and flagellar beat frequency. CASA Measures the parameters such as VCL, VSL and amplitude of lateral head (ALH). The quality of sperm movement is based on a classification system of 0 to 4, wherein 0 represents no movement and 4 represents

excellent forward progression for example, a semen sample with 60% motility would be characterized as 3+ to 4.2.

Non-surgical factors related to male infertility

Gonado-toxine	Ejaculatory dysfunction
Genital tract infection	Hormonal imbalances
Coital timing	Testicular hyperthermia

Retrograde ejaculation

Disruption of the innervations of the vasa deferentia and bladder neck can result in retrograde ejaculation. Diabetes mellitus complicated by peripheral neuropathy, multiple sclerosis, medical therapies interfering with sympathetic tone, Tran urethral resection of the prostate bladder neck surgery retroperitoneal lymph node dissections and extensive pelvic surgery also can lead to retrograde ejaculation.

The diagnosis is confirmed by identification of large numbers of sperm in a post ejaculate urine specimen.

Microbiology

The presence of white blood cells (WBC's) in the patient's semen may indicate an infection. Accordingly the semen should be evaluated for bacterial growth, mycoplasma and chalmydia.

Distribution of final diagnostic categories found in male fertility clinic diagnosis

Varicocele	Endocrine
Testicular failure	Cryptorchidism
Ejaculatory Failure Agglutination	Low Volume
Sexual Dysfunction Viscosity	Idiopathic

Necrospermia

Relative frequency of causes and associated conditions in men who present with infertility.

Conditions

- Varicocele
- Viral Orchitis
- Immotile sperm
- Coital disorders
- Abdrogen resistance
- Radiation/chemotherapy
- Obstruction of epididymis or of vas deferens
- Klinefelters syndrome

Surgical operations in the male possibility associated with male infertility

1. Hydrocele
2. Varicocele
3. Inguinal hernia
4. Vasectomy
5. Prostatectomy
6. Sympathectomy
7. Testicular torsion
8. Hypospadias
9. Urethral strictures or diverticula
10. Bladder neck operation

WHO manual – semen analysis – nomenclature's

1. Normozoospermia	-	Normal semen
2. Aspermia	-	Absence of ejaculation
3. Azoospermia	-	Absence of sperms in the semen
4. Oligo zoospermia	-	Less than 20 millions count / ml
5. Astheno zoospermia	-	Less than 5.0% spermatozoa with Forward Progression
6. Terato zoospermia	-	Less than 30% spermatozoa with Normal head morphology
7. Oligoastheno teratozoosperima	-	Signifies the disturbance of all the above variables

Sub fertility options – Abbreviations:

For infertility disorders there are so many latest and advanced techniques are available and some of them are listed below,

IVF	**:**	In Vitro Fertilization
GIFT	**:**	Gamete Intra Fallopian Transfer
TET	**:**	Tubal Embryo Transfer
TUFT	**:**	Trans-Uterine Fallopian Transfer
ICSI	**:**	Intra-Cytoplasmic Sperm Injection
SUZI	**:**	Sub Zonal Sperm Injection (Directly into Ovum)
ZIFT	**:**	Zygote Intra-Fallopian Transfer
POST	**:**	Peritoneal Oozyte Sperm Transfer
PESA	**:**	Percutaneous Epididymal Sperm Aspiration
MESA	**:**	Micro Epididymal Sperm Aspiration –from testis

Sperm Function Tests

Sperm migration test	:	More than 150 million in 250 micl - Fertile
Hypo osmotic test	:	More than 60% with HOS positive - Fertile

Acrosomal intactness

Test nuclear chromatin	:	More than 60% with halo 30 micm - Fertile
Decondensation test	:	More than 70% NCD – Fertile

Sperm mitochondrial

Activity Index	:	More than 50 SMAI – Fertile

In vitro cervical mucus

Penetration test	:	Score: 0-Negative, 3-Poor, 6-Good, 9-Excellent
Post coital test	:	More than 10 rapid forward progressing Sperms/HPF
Post ejaculatory urine	:	No spermatozoa in urine

3.3. PHARMACOLOGICAL REVIEW

Pharmacological study of anti-fertility activity in animal models

In vivo methods

Emergent spermatozoa made non functional/oligospermia/aspermia.

1. **Fertility test**

Evaluation of average litter anti-fertility agents negatively affects the average litter size. Groups of 5-10 male rats of proven fertility are treated with drug and are paired with fertile female in ratio of 1:3. Daily vaginal smears are examined for presence of sperms. All females passed through estrus cycle must have mated animals are kept separately till the completion of gestational period. The litters are counted. Average litter size: Total number of litters/number of females mated. If vaginal smear shows leukocytes for 10-14 days, pseudo pregnancy is confirmed. If insemination is not detected then inhibition of libido or aspermia copulation might be cause. Fertility pattern can be obtained from changes in average litter size[41].

2. **Androgenic activity**

Chicken comb method

This assay is based in principle of growth of capon comb by androgenic compounds. This method has been useful for isolation and structural elucidation of natural androgens. In the beginning of assay, the sum of length plus height of each individuals comb is determined by measurement with a millimeter rule placed directly on the comb. The capons are injected daily i.m. for 5 consecutive days with a solution or suspension of test compound or the standard in 1 ml olive oil. After 24 hr the last injection the comb is re-measured and groth of comb is expressed as the sum of length and height in millimeter. Group of 8 animals are used for at least 2 doses of test compound. The lot of control and test group is compared with suitable statistical analysis[41A].

3. Anti androgenic activity

Chicken comb method

Inhibition of growth of capon comb by anti androgenic compounds. One or 3 days old male or female white leghorn chicks are housed at constant temperature in heated incubator. Testosterone is incorporated into the finally ground chick starting mash at concern of 80 mg/kg food. Chicks are placed on this diet on day 1. The test compound is dissolved in sesame oil. Each day for 4 days 0.1 ml of the oil solution is injected s.c. Control chicks receive only the vehicle. 24 hr after the last injection the animals are sacrified, the combs removed and after blotting of cut edge, weighed rapidly to the nearest 0.5 mg. The weight of control and test groups are compared using suitable statistical method. In a shaking bath maintained at 25° C, 50 ml^3 H pyrilamine (2×10^{-9} m), 50 ml membrane suspension from guinea pig whole brain (10 mg/ml) per sample are incubated for 30 min[41B].

4. Ethanol induced

Adult male Wistar rats weighing 180-210 gms were used for this study. The animals were subjected under standard photoperiodic condition of 12:12 hrs light: dark cycle. The animals were fed with standard rodent pellet and water *ad libitum*. Animals were acclimatized to laboratory conditions one week prior to initiation of experiments. The animals were randomly selected and divided into four groups (I, II, III and IV) of six rats (n=6) each. Individual identification of the animal was made by marking. Group I animals served as control and received only 1ml milk, p.o. for 60 days. *Group II* served as negative control (Alcohol induced), received 0.5 ml of 25% ethanol /kg /BW /day for 60 days. Experimental groups splits into group III and IV served as the treated groups and received trial drug which was grounded in mortar-pestle with milk. *Group III* was treated with 25% of 0.5 ml ethanol /kg/BW/day along with 100mg/kg of trial drug orally once for 60 days. *Group* IV was treated with 25% of 0.5 ml ethanol /kg/BW/day along with 200mg/kg of trial drug orally once for 60 days Administration was done once a day by oral gavage in the morning.

5. Cohabitation test

This test determines the time interval for litter production after placing treated males with 2 females each. The date of mating is calculated from the date of

parturition. This method is suitable for drugs known to cause sterility for several weeks. Adult female and male albino rats of proven fertility are used for the study. They are kept for mating in the ratio of 2:1 till both females deliver litters. The date of mating is calculated from the date of parturition. The time interval for litter production after placing treated males with two females is calculated[42A].

6. Subsidiary test

This test determines the changes in spermatozoa count with time. The anti-fertility drugs affect the spermatozoa count negatively. Adult male albino rats weighing between 150-250 gm are used for the study. They are kept in a cage containing artificial or animal vagina. The vagina is artificially simulated by a cylindrical plastic jacket with a rubber liner filled with water at 5° C, 0.5 ml of ejaculate is diluted with saline containing traces of formalin. The resulting suspension is counted on a haemocytometer[42B].

7. Slidenafil citrate

The adult male rats were randomly selected and divided into three groups with six animals in each group. Group I served as control and received 2ml of milk as oral daily for 21 days. Group II served as standard and received 2ml of milk as oral daily for 20 days then on 21st day, slidenafil citrate 0.7 mg/kg was given orally. Group III received 150 mg of trial drug suspended in 2 ml of milk as oral daily for 21 days.

Then on 21st day three hours following the administration, for the evaluation of aphrodisiac activity, these rats were individually placed in cages and were given 10 min adaptation periods. A separate female rat in Oestrus stage was taken for each male. The following parameters of sexual behaviour were monitored 15 min after pairing, under dim light. Latency of first mount, number of mounts, latency of first intromission, inter intromission interval, number of intromissions, latency of first ejaculation, latency of second ejaculation, average ejaculation latency and number of ejaculations was recorded.

3.4. PHARMACEUTICAL REVIEW

Chooranam

Definition

Chooranam is a fine powder of drugs. The "*Chooranam*" may be applied to powder of a single drug or a mixture of two or more drugs, which are powdered separately prior to their being mixed to homogeneity. (Formulary Of Siddha Medicine, 1993)

Method of preparation

Equipment required

1. The drug enumerated in the recipe in clean and well dried state.
2. A mortar and pestle.
3. A fine sieve or fine cloth of close mesh.

Process of preparation

The drugs which are to be used in the preparations should be taken from recently collected material. Drugs which are aged by prolonged storages or changed in colour, taste and odour, and those that are insects infested or attacked by fungi should be positively rejected.

However drugs like Embelia fruits, Senna, Long Pepper, Jaggery and cows ghee are preferred from fairly aged stock, provided they are not infested with pests, deteriorated or spoiled or developed racidity.

In general the aromatic drugs are slightly fried in order to enhance their aroma and milling properties. Any extraneous material, organic or inorganic, should be removed from the drugs by close inspection.

The *Chooranam* should be so fine to be called amorphous and should never be damp. The fineness of the sieve should be 100 mesh or still finer.

Purification of the prepared *Chooranam*

"தானென்ற சூரணத்தின் சுத்திக்கேளு
தப்பாதே சரக்கெல்லாஞ் சூரணித்து
நானென்ற வாவின் பாலாற் பிசைந்து
நலமான சட்டியிலே பாலைவிட்டு
வானென்ற சுத்தசலம் பாதிவிட்டு
வளமாக மேற்சீலை கோடு கட்டிப்
பானென்ற சூரணத்தைப் பிட்டுபோல் வைத்து
பதறாதே வெந்தெடுக்கச் சித்தியமே"

-அகஸ்தியர் வைத்திய இதத்தினச் சுருக்கம்

The prepared *Chooranam* is mixed with the milk in a pot half a quantity milk and half a quantity water is taken. The mouth of the pot is covered with a thin cloth material. Above this cloth the mixed *Chooranam* is placed. The pot is placed over the stove and heated.

"ஆமப்பா ரவியுலர்த்திப் பொடிதான் செய்து
அப்பனே சமனாய்ச் சர்க்கரையைச் சேர்த்து
நாமப்பா கொண்டு வர தோஷம் போச்சு
நன்நாகச் சுத்தி செய்யாச் சூரணந்தான்
தாமப்பா ரோகத்தை வெல்லா தப்பா
தளமான வியாதி யெல்லாம் பாரிக்கும் பார்
வேமப்பா சுத்தி செய்து கொண்டாயானால்
வெகுசுறுக்காய் தீருமா வியாதி கேளு"

-அகஸ்தியர் வைத்திய இதத்தினச் சுருக்கம்

Then the *Chooranam* is placed in the sunlight and powdered. Equal amount of sugar is added and taken internally. All type of diseases get cured. If the drug is taken without purification the disease does not cure. If taken after purification the disease cures easily.

Storage

The prepared *Chooranam* should be allowed to cool by spreading and mixing, prior to packing. They should be stored in tightly stoppered glass, polythene or tin containers, or in polythene or cellophane bags and sealed. These bags should in turn be enclosed in cardboard boxes.

The *Chooranam* to facilitate easy handling and to assure exact dosage administration, could be pressed into tablets, could be packed in bottles or tubes made either of glass or plastic or packed in strip of metal foil or plastic sheets.

In industry the tablets are made, counted & packed by electronic devices.

Then *Chooranam* is said to retain its potency for 2 months and then gradually deteriorate. However if properly packed & stored they keep good for a year. (Formulary of Siddha Medicines, 1993)

According to AYUSH guidelines shelf life of *Chooranam* is one year.

Table No. 3. Analytical Specifications of *Curna/Choornam* [43]

Sl.No	TESTS
1.	Description Macroscopic, Microscopic
2.	Loss on drying at 105^0 C
3.	Total – ash
4.	Acid – insoluble ash
5.	Water-soluble extractive
6.	Alcohol – soluble extractive
7.	Particle size (80-100 mesh for Churna; 40-60 mesh for churna)
8.	Identifications, TLC/HPTLC-with marker (wherever possible)
9.	Test for heavy/Toxic metals Lead Cadmium

Sl.No	TESTS
	Mercury
	Arsenic
10.	Microbial contamination
	Total bacterial count
	Total fungal count
11.	Test for specific Pathogen
	E. coli
	Salmonella spp.
	S.aureus
	Pseudomonas aeruginosa
12.	Pesticide residue
	Organochlorine pesticides
	Organophosphorus pesticides
	Pyrethroids
13	Test for Aflatoxins (B1,B2,G1,G2)

3.5. LATERAL RESEARCH

3.5.1. *Curculigo orchioides*

Anti hyperglycaemic activity[44]

The ethanolic extract of *Curculigo orchioides* rhizomes, given orally at a dose of 100 mg/kg b.w., possesses significant hyoglycemic in both normal and glycose loaded rat. Also in diabetic induced rats significantly.

Anti bacterial activity[45]

The essential oil fraction from *Curculiogo orchioides* possesses significant ($P < 0.001$) antibacterial activity ar very low concentration (20 g/disc) against the pathogenic Gram positive S. aureus (CI) bacteria.

Estrogenic activity[46]

The ethanolic extract of rhizomes of *Curculigo orchioides* at three different doses (viz., 300, 600, 1200 mg/kg body weight). Alcoholic extract of *Curculigo orchioides*

showed a significant increase in percentage vaginal cornification, uterine wet weight ($P < 0.001$), uterine glycogen content ($P < 0.001$) and a proliferative changes in uterine endometrium compared to the control.

3.5.2. *Tribulus terrestris*

Anti diabetic activity[47]

50 mg/kg body wt. Concentration of methanolic extract of *T. terrestris* produces significant decrease in blood level, after 4 and 6 hours of treatment as compared to untreated diabetic mice. After 4 and 6 hours of treatment, the percent of reduction in blood glucose level produced by *T. terrestris* (43±4.1), (41±3.4). After three weeks of treatment, blood glucose level in diabetic mice treated with *T. terrestris* decreases (60%) to below normal level.

Anti urolithiatic activity[48]

The ethanol extract of T.terrestris(Fruit) was tested for activity against artificially induced urolithiasis in albino rats. The extract was administered at daily oral doses of 25, 50 and 100 mg/kg for 4 months. It exhibited dose-dependent antiurolithiatic activity and almost completely inhibited stone formation.

3.5.3. *Mucuna pruriens*

Antioxidant activity[49]

The methanol extract of *Mucuna pruriens* (MEMP) seeds strong antioxidant activity by inhibiting DPPH and hydroxyl radical, nitric oxide and superoxide anion scavenging, hydrogen peroxide scavenging, and reducing power activities when compared with different standards such as BHT, L-Ascorbic acid, Curcumin, Quercetin, and α-tocopherol. In addition, the MEMP found to contain a noticeable amount of total phenols, which play a major role in controlling antioxidant. The results of this study show that the MEMP can be used as easily accessible source of natural antioxidanrs and as a possible food supplement or in pharmaceutical industry. However, the components responsible for the antioxidant activity of MEMP are currently unclear. Therefore, it is suggested that further works should be performed on the isolation and identification of the antioxidant components in MEMP.

Immunomodulatory activity[50]

Mucuna pruriens seed extract at 100 mg and 200 mg/kg p.o, significantly inhibited the adhesion of neutophils to nylon fibers which stimulates the process of margination of cells in the blood vessels. This indicates that at the site of inflammation, *Mucuna pruriens*, seeds reduces the number of neutrophils, thus decreasing their phagocytic action and the release of various enzymes and mediators that make inflammation worse. *Mucuna pruriens* seed extract at 100 mg/kg were found to suppress delayed type hypersensitivity reaction induced by SRBCs in mice. The decrease in delayed type hypersensitivity reactions revealed to the inhibitory effect of drug in T-lymphocyctes and cell types required for expression of humoral response to SRBCs, as evidenced by mark decrease in haemagglutination titres in mice was also observed.

3.5.4. *Hygrophila auriculata*

Hepatoprotective activity[51]

The antihepatotoxic effect of the total alkaloid fraction was observed in freshly isolated rat hepatocytes at very low concentrations (80-40 µg-ml). A dose-dependent increase in the percentage viability was observed when CCl_4-exposed HepG2 cells were treated with different concentrations of the total alkaloid fraction. Its in vivo hepatoprotective effect at 80 mg/kg body weight was comparable with that of the standard Silymarin at 250 mg/kg body weight.

Antidiabetic activity[52]

Treatment of diabetic rats with aerial parts of *Hygrophila auriculata* extract (HAEt, 100 and 250 mg/kg body weight) for 3 weeks showed significant reduction in blood glucose, thiobarbituric acid reactive substance (TBARS) and hydroperoxide in both liver and kidney. The treatment with HAEt significantly increased the glutathione (GSH), glutathione peroxidase (GPx), glutathione S-transferase (GST) and catalase (CAT) in the drug-treated group, which is comparable to the control group. HAEt and glibenclamide-treated rats also showed decreased lipid peroxidation that is associated with increased activity of superoxide dismutase (SOD) and catalase.

MATERIALS AND METHODS

4. MATERIALS AND METHODS

4.1 Preparation of the drug

Drug selection

In this research work, the "***Thathu Viruthi Chooranam",*** a poly herbal formulation, has been selected to evaluate ***Thathu Balaveenam*** (Oligospermia), mentioned in "***Sarabenthirar Vaithiya Rathnavali".***

Ingredients

Nilapanai kizhangu (*Curculigo orchioides*)	-	1 *palam* (35 gram)
Nerunjil vaer (*Tribulus terrestris*)	-	1 *palam* (35 gram)
Poonaikaali vithai (*Mucuna pruriens*)	-	1 *palam* (35 gram)
Neermulli vithai *(Hygrophila auriculata)*	-	1 *palam (35* gram*)*
Nilapanai samoola saaru	-	*50 ml*

Source of Collection:

Three of the drugs were purchased from authorized country Raw Drug Store in Parry's corner, Chennai and the *Nerunjil* plant (whole) was collected in the campus of Government Siddha Medical College, Chennai.

Identification and Authentication of the drug

The collected raw materials and plants were identified and authenticated by Botanist and faculties of *Gunapadam* Department, Government Siddha Medical College, Chennai, Tamilnadu.

(Ref No: GSMC/PGGM/068-071/2014-2017)

Purification of the ingredients

Nilapanai kizhangu

Tuber of *Nilapanai kizhangu* (*Curculigo orchioides*) was washed in tap water and the soil and impurities were removed.

Nerunjil vaer

Roots of *Nerunjil* (*Tribulus terrestris*) were cleaned well without any dust and impurities.

Poonaikaali vithai

Seeds of *Mucuna pruriens* were washed thoroughly then boiled with 100 ml of milk and outer skin was removed.

Neermulli vithai

Seeds of *Hygrophila auriculata* were cleaned well without any dust and impurities.

Preparation of *Thathu Viruthi Chooranam*

The ingredients after purification were ground separately to powder. The powder was sieved through a white cloth (***Gunapadam Thathu Jeeva Vagupu*)**. The powder were ground with the juice of the whole plant *Nilapanai* (*Curculigo orchioides*) in stone mortar. This process is continued till all the juices gets dried. It is ground againted to dry, the prepared test drug was stored in a clean, air tight glass container.

Drug name	**:**	*Thathu Viruthi Chooranam*
Dosage	**:**	1-2 Grams twice a day- after food
Indication Sexual	**:**	Spermatorrhoea, Impotency, Nervous weakness & tonic.
Vehicle	**:**	Milk
Colour	**:**	Brown
Date of manufacture	**:**	14 March 2017
Date of expiry	**:**	13 June 2017
Reference	**:**	*Sarabenthirar Vaithiya Rathnavali,* Page no-474.

Administration of drug

Form of the medicine	:	*Chooranam* (Powder)
Route of Administration	:	Oral
Dose	:	1-2 grams
Anubanam (Vehicle)	:	Milk
Time of Administration	:	Twice a day;

Ingredients

Fig 1.1: *Curculigo orchioides*

Fig 1.2: *Tribulus terrestris* (Root)

Fig 1.3: *Mucuna pruriens* (Seed)

Fig 1.4: *Hygrophila auriculata* (Seed)

4.2. Standardization of the drug

Standardization of herbal formulations is essential to assess quality of drugs[53]. And also it is necessary to conduct uniform rules for preparing drug[54]. Standardization of the drug brings the validation to be used as a medicine by subjecting the drug TVC to many analysis and determining its quality and effectiveness. Standardization includes many studies such as its organoleptic properties, physical characteristics and phytochemical properties and also to assess the active principles and elements present in the drug. Thus standardization brings the efficacy and potency of the drug.

4.2.1. Physicochemical analysis

Organoleptic character

The organoleptic characters of the sample drug were evaluated.1gm of TVC was taken and the colour, texture, particle size and other morphology were viewed by naked eye under sunlight. Then the result was noted.

pH value

Potentiometrically pH value determined by a glass electrode and a suitable pH meter.

Loss on Drying

The powdered drug is dried in the oven at 100- 105°C to constant weight.

Determination of Ash Values[55]

Total Ash

Accurately weighted 3g of *Thathu Viruthi Chooranam* was taken and incinerated in a crucible dish at a temperature not more than 450^{o}C until free from carbon. Then it was cooled and weighed. The percentage of ash was calculated with the reference to the air-dried drug.

4.2.2. Phytochemical analysis

The *Thathu Viruthi Chooranam* (10g) was extracted with the solvents namely methanol, ethyl acetate and chloroform. The extracts were filtered and the

concentrated under vacuum, followed by drying (40°C). The extracts were screened for the presence of phytochemicals like alkaloids, flavonoid, carbohydrates, glycosides, saponins, tannins and triterpenoids by standard methods. The compositions of these phytoconstituents depend upon the nature of the drug and the solvent used. It also gives an indication whether the crude drug is exhausted or not[56].

Results were discussed in table no. 9

4.2.3. TLC/ HPTLC finger print studies

HPTLC finger printing was carried out as per the reference.

Preparation of spray reagent-vanillin-sulphuric acid reagent

Vanillin (1g) was dissolved in ice cold ethanol (95ml). Add to 5ml of cooled concentrated sulphuric acid. Ice was added and stirred well. The solution was stored in refrigerator.

Chromatographic conditions

Instrument : CAMAG (Switzerland).

Sample applicator : Camag Linomat - IV applicator with N_2 gas flow.

Photo documentation System : Digi store - 2 documentation system with Win Cat and video scan software.

Scanner : Camag HPTLC scanner - 3 (030618), Win Cats - IV.

Development Chamber : Camag HPTLC 10X10, 10 X 20 twin trough linear development chamber.

Quantity applied : 5, 10 µl for extracts and 5 µl for standards

Stationary phase : Aluminium plate pre-coated with silica gel 60 (E.Merck)

Plate thickness : 0.2 mm.

Mobile Phase : Chloroform extract - Toluene: Ethyl acetate (9:2).

Scanning wavelength : 254 nm

Laboratory condition : 26 ± 5°C and 53 % relative humidity

The plate was developed up to a height of 8 cm, air dried, spots were observed under the UV light at 254 and 366 nm. Finally the plates were derivatized using vanillin-sulphuric acid reagent heated at 105° till colour spots appeared.

Results were discussed in table no. 10, 11

4.2.4. Biochemical analysis

Methodology for chemical analysis

Preparation of extract

5gm of TVC was weighed accurately and placed in a 250ml clean beaker and added with 50ml of distilled water. Then it was boiled well for about 20 minutes. Then it was cooled and filtered in a 1000ml volumetric flask and made up to 100ml distilled water.

Table No.4. Test for basic radicals

PROCEDURE	OBSERVATION	INFERENCE
Test for Potassium: A pinch of sample is treated with 2ml of Sodium nitrate solution and then treated with 2ml of Cobalt nitrate in 30% of glacial acetic acid.	Formation of Yellow colour precipitate	Presence of Potassium
Test for Calcium: Taken 2ml of extract in a clean test tube. Then Acetic acid and Potassium chromate solution were added.	Formation of Yellow Precipitate	Presence of Calcium
Test for Magnesium: 2ml of extract was taken in a clean test tube, few drops of Magnason reagent was added in drops.	Formation of Blue colour precipitate	Presence of Magnesium
Test for Ammonium: 2ml of extract was taken in a test tube and added few ml of Nessler's reagent.	Appearance of Brown colour	Presence of Ammonium

PROCEDURE	OBSERVATION	INFERENCE
Test for Sodium: 2 pinches of TVC as mixed with HCL and made it into paste and introduced into the blue flame of Bunsen burner.	Appearance of intense Yellow colour	Presence of Sodium
Test for Iron (Ferrous): 2ml of extract was taken in a clean dried test tube and conc.HNO and Ammonium thiocyanate were added.	Appearance of Blood red colour	Presence of Ferrous iron
Test for Zinc: 2ml of the extract was taken in a test tube and Potassium ferro cyanide solution was added.	Formation of White colour precipitate	Presence of Zinc
Test for Aluminium: To the 2ml of the extract was taken in a test tube Sodium hydroxide drops were added to it.	White precipitate obtained	Presence of Aluminium
Test for Lead: 2ml of extract was taken in a test tube and added with 2ml of Potassium iodide solution.	Formation of Yellow colour precipitate	Presence of Lead
Test for Copper: To a small portion of a extract dilute hydrochloric acid was added and then Hydrogen sulphide gas is passed through the solution.	Black precipitate obtained	Presence of Copper
Test for Mercury: 2ml of the extract was taken in a test tube and treated with 2ml of Sodium hydroxide solution.	Formation of Yellow precipitate	Presence of Mercury
Test for Arsenic: 2ml of the extract was taken in a test tube and treated with 2ml of Sodium hydroxide solution.	Formation of Brownish red precipitate	Presence of Arsenic

Results were discussed in table no: 12

Test for acid radicals

Table No.5. Test for acidic radical

PROCEDURE	OBSERVATION	INFERENCE
Test for Sulphate: 2ml of the extract was taken in clean, dry test tube and 5% barium chloride solution was added to it.	Formation of white precipitate	Presence of Sulphate
Test for Chloride: The extract was taken in a test tube and then treated with Sliver nitrate solution.	Formation of White Precipitate	Presence of Chloride
Test for Phosphate: The extract was taken in a test tube and treated with ammonium molybdate and Conc.HNO_3.	Formation of Yellow colour precipitate	Presence of Phosphate
Test for Carbonate: The substance was taken in a clean dry test tube and then treated with Conc.HCL.	Formation of Effervescence	Presence of Carbonate
Test for Fluoride and Oxalate: 2ml of extract was taken in a test tube and added with 2ml of dil.acetic acid, 2ml calcium chloride solution and then heated.	Formation of cloudy appearance	Presence of Fluoride and Oxalate
Test for Nitrate: 1gm of the TVC was heated ith copper turnings and Conc.H_2SO_4 and observed the test tube vertically down.	Characteristic changes	Presence of Nitrate

Results were discussed in table no: 13

4.2.5. Availability of microbial load

Enumeration of bacteria by plate count – agar plating technique[57].

The plate count technique is one of the most routinely used procedure because of the enumeration of viable cells by this method.

Principle

This method is based on the principle that when material containing bacteria is cultured, every viable bacterium develops into a visible colony on a nutrient agar medium. The number of colonies, therefore are the same as the number of organisms contained in the TVC.

Dilution

A small measured volume of TVC are mixed with a large volume of sterile water called the diluent or dilution blank. Dilution are usually made in multiples of ten. A single dilution is calculated as follows

$$\text{Dilution} = \frac{\text{Volume of the sample}}{\text{Total volume of the sample and the diluent}}$$

Requirements

- Sample or Bacterial suspension
- 9 ml dilution blanks (7)
- Sterile petri dishes (12)
- Sterile 1 ml pipettes(7)
- Nutrient agar medium (200 ml)

Procedure

1. Label the dilution blanks as 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}.
2. Prepare the initial dilution by adding 1 ml of the TVC extract into a 9 ml dilution blank labelled 10^{-1} thus diluting the sample 10 times.
3. Mix the contents by rolling the tube back and forth between hands to obtain uniform distribution of organisms.
4. From the first dilution transfer 1 ml of the suspension while in motion, to the dilution blank 10^{-2} with a sterile and fresh 1 ml pipette diluting the original specimen to 100 times.
5. From the 10^{-2} suspension, transfer 1 ml of suspension to 10^{-3} dilution blank with a fresh sterile pipette, thus diluting the original sample to 1000 times.

6. Repeat this procedure till the sample have been diluted 10,000,000 times using every time a fresh sterile pipette.
7. From the appropriate dilutions transfer 1ml of suspension while in motion, with the respective pipettes, to sterile petri dishes. Three petri dishes are to used for each dilution.
8. Add approximately 15 ml of the nutrient medium, melted and cooled to 45^0c, to each petri dish containing the diluted TVC extract. Mix the contents of each dish by rotating gently to distribute the cells throughout the medium.
9. Allow the plates to solidify.
10. Incubate these plates in an inverted position for 24-48 hours at 37^0c.

Observation

Observe all the plates for the appearance of bacterial colonies. Count the number of colonies in the plates.

Calculate the number of bacteria per ml of the original suspension as follows:

$$\text{Organisms per millimetre} = \frac{\text{Number of colonies (average of 3 repleates)}}{\text{Amount of plated} \times \text{dilution}}$$

4.2.6. Sophisticated instrumental analysis

FT-IR (Fourier Transform Infra-Red Spectroscopy)

Fig No.3. Image of FTIR Analyser

FT-IR spectra were recorded at SAIF, IIT Madras, India. The Perkine Elmer Spectrum One Fourier Transform Infrared (FTIR) Spectrometer was used to derive the FT IR Spectra of *Thathu Viruthi Chooranam* in Potassium Bromide (KBr) matrix with scan rate of 5 scan per minute at the resolution $4cm^{-1}$ in the wave number region $450\text{-}4000cm^{-1}$. The samples were grounded to fine powder using agate motor and pestle and the mixed with KBr[58]. They were then Pelletized by applying pressure to prepare the specimen (the size of specimen about 13 mm diameter and 0.3 mm in thickness) to recorded the FT- IR Spectra under Standard conditions. FT- IR Spectra were used to determine the presence of the functional groups and bands in the *Thathu Viruthi Chooranam*. The recorded spectrum shows in figure.

SEM (Scanning electron microscope)

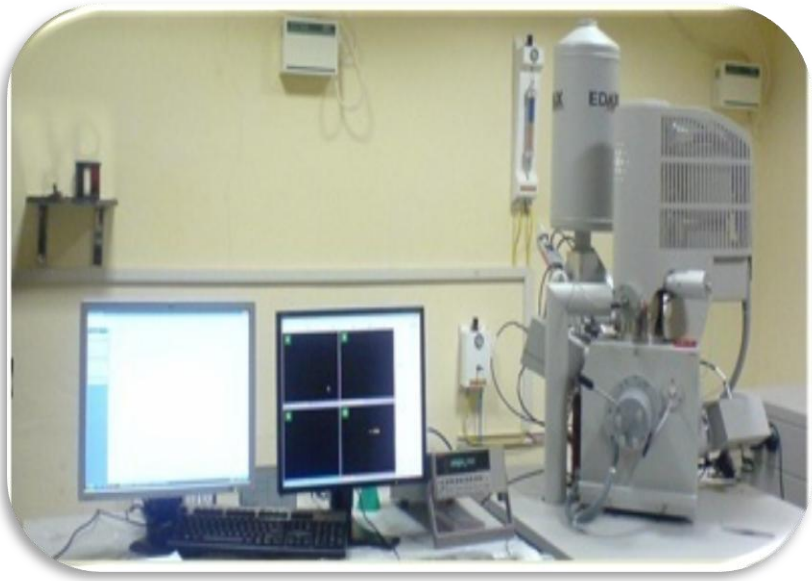


Fig No.4. Image of SEM Analyser

To evaluate the size of the particle, surface topography SEM analysis was carried out using at SAIF, IIT madras.The sample was mounted on specimen stub, placed inside the microscope's vacuum column evaporator and a beam of electrons passed from an electron gun, travelled through a series of magnetic lenses. The electrons are counted by the detector and the signals are sent to the amplifier. The number of electrons dispersed from each spot of the sample builds up the resultant image. The micrographs obtained give sufficient data about the topography of the sample.

XRD (X-RAY POWDER DIFFRACTION)

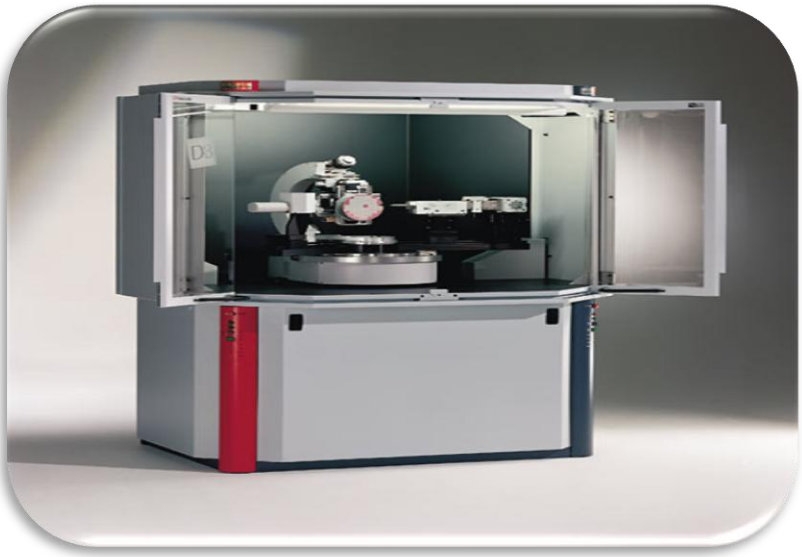


Fig No.5. Image of XRD Analyser

The XRD studies were done in IIT madras by using Bruker discover D8 X ray diffractometer.

Definition

X-ray powder diffraction is most widely used for the identification ofunknown crystalline materials (e.g. minerals, inorganic compounds). Determination of unknown solids is important to studies in geology, environmental science, material science and biology.

Applications

- Characterization of crystalline materials
- Identification of fine-grained minerals such as clays and mixed layer clays that are difficult to determine optically
- Determination of unit cell dimensions.

With specialized techniques, XRD can be used to

- Determine crystal structures using Rietveld refinement
- Determine of modal amounts of minerals (quantitative analysis)
- Characterize thin films samples by:

- determining lattice mismatch between film and substrate and toinferring stress and strain
- determining dislocation density and quality of the film by rockingcurve measurements
- measuring super lattices in multilayered epitaxial structures
- determining the thickness, roughness and density of the film usingglancing incidence X-ray reflectivity measurements
- Make textural measurements, such as the orientation of grains, in apolycrystalline sample.

Strengths and Limitations of X-ray Powder Diffraction

Strengths

- Powerful and rapid (< 20 min) technique for identification of an unknownmineral
- In most cases, it provides an unambiguous mineral determination
- Minimal sample preparation is required
- XRD units are widely available
- Data interpretation is relatively straight forward.

Limitations

- Homogeneous and single phase material is best for identification of unknown
- Must have access to a standard reference file of inorganic compounds
- Requires tenths of a gram of material which must be ground into a powder
- For mixed materials, detection limit is ~ 2% of sample
- For unit cell determinations, indexing of patterns for non-isometric crystalsystems is complicated.

Sample Collection and Preparation

Determination of an unknown requires: the material, an instrument for grinding, and a sample holder.

- Obtain a few tenths of a gram (or more) of the material, as pure as possible
- Grind the sample to a fine powder, typically in a fluid to minimize inducing extra strain (surface energy) that can offset peak positions, and to randomizeorientation.

- Powder less than ~10 μm(or 200-mesh) in size is preferred place into a sample holder or onto the sample surface.

ICPOES (INDUCTIVELY COUPLED PLASMA OPTIC EMISSION SPECTROMETRY)

Fig No.6. Image of ICP-OES Analyser

The Inductively Coupled Plasma Optical Emission Spectrometric (ICP-OES) analysis was done using Perkin Elmer Optima 5300 DV.

Sample preparation

Inductively Coupled Plasma Spectroscopy techniques are the so-called "wet" sampling methods whereby TVC are introduced in liquid form for analysis.

100 mg TVC was occupied in a clean, dry test tube. To this, 3 ml Nitric acid was added and mixed well and allowed for few minutes untill the reactions were completed. And then, 25 ml of Refined water, was added to prepare digested solution. The digested TVC solution was shifted into plastic containers and labeled properly. It was completed in Bio-chemistry lab, Govt. Siddha Medical College, Chennai-106.

4.3. TOXICOLOGICAL STUDIES

4.3.1. Acute toxicity – OECD guidelines - 423[59]

Introduction

The acute toxic class method was a stepwise procedure with the use of 3 animals of a single sex per step. Depending on the mortality and the moribund status of the animals, on average 2-4 steps may be necessary to allow judgment on the acute toxicity of the test substance. Morbid animals or animals obviously in pain or showing signs of severe and enduring distress shall be humanely killed, and are considered in the interpretation of the test results in the same way as animals that died on test. The method allows for the determination of an LD50 value only when at least two doses result in mortality higher than 0% and lower than 100%.

Acute toxicity study was carried out as per OECD guideline (Organization for Economic Co - operation and Development, Guideline-423.

The experimental protocol was approved by the institutional ethical committee (IAEC) under CPCSEA.

Animal : Healthy Wistar albino female rat weighing 200–220 gm

Principle

It was the principle of the test that based on a stepwise procedure with the use of a minimum number of animals per step, information was obtained on the acute toxicity of the test substance to enable its classification. The substance was administered orally to a group of experimental animals at one of the defined doses. The substance was tested using a stepwise procedure, each step using three animals of a single sex. Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step, i.e.; − no further testing was needed − dosing of three additional animals with the same dose − dosing of three additional animals at the next higher or the next lower dose level. The method will enable a judgment with respect to classifying the test substance to one of a series of toxicity classes. Studied carried out at three female rats under fasting condition, signs of toxicity was observed for every one hour for first 24 hours and every day for about 14 days from the beginning of the study.

Methodology

Selection of animal species

The preferred rodent species was rat, although other rodent species may be used. Healthy young adult animals of commonly used laboratory strain Swiss albino rat were obtained from Animal house of king's institute, Guindy, Chennai. Females should be nulliparous and non-pregnant. Each animal at the commencement of its dosing should be between 8 and 12 weeks old and its weight should fall in an interval within±20 % of the mean weight of the animals. The studies were conducted in the animal house of C.L.BaidMetha College of pharmacy, Thuraipakkam, Chennai.

Housing and feeding conditions

The temperature in the experimental animal room should be 22ºC (+3ºC). Although the relative humidity should be at least 30% and preferably not exceed 70% other than during room cleaning the aim should be 50-60%. Lighting should be artificial, the sequence being 12 hours light, 12 hrs dark. For feeding, conventional laboratory diets may be used with an unlimited supply of drinking water. Animals may be grouped and tagged by dose, but the number of animals per cage must not interfere with clear observations of each animal.

Preparation of animals

The animals are randomly selected, marked to permit individual identification, and kept in their cages for at least 7 days prior to dosing to allow for acclimatization to the laboratory conditions.

Experiment procedure

Administration of doses

TVC was prepared as per the classical Siddha literature was suspended in 2% CMC with uniform mixing and was administered to the groups of Wistar albino rats. It was given in a single oral dose by gavages using a feeding needle. Animals were fasted prior to dosing. Following the period of fasting, the animals were weighed and then the test substance was administered. After the substance has been administered, food was withheld for a further 3-4 hours. The principle of laboratory animal care was

followed. Observations were made and recorded systematically and continuously observed as per the guideline after substance administration.

The visual observations included skin changes, mobility, aggressively, sensitivity to sound and pain, as well as respiratory movements. They were deprived of food, but not water 16–18 hours prior to the administration of the test suspension. Finally, the number of survivors was noted after 24 hours and these animals were then maintained for a further 14 days and observations made daily. The toxicological effect was assessed on the basis of mortality.

Number of animals and dose levels

Since this TVC has been under practice for long time and likely to be non-toxic, a limit test at one dose level of 2000 mg/kg body weight will be carried out with 6 animals (3 animals per step).

Duration of Study : 48 hours

Evaluation : 14 Days

Limit test

The limit test was primarily used in situations where the experimenter has information indicating that the test material was likely to be nontoxic, i.e., having toxicity only above regulatory limit doses. A limit test at one dose level of 2000 mg/kg body weight was carried out with three animals per step. The test substance-related mortality was not produced in animals, so further testing at the next lower level need not be carried out.

Observations

- The animals were observed individually after dosing at least once during the first 30mins and periodically during the first 24 hours.
- Special attention: First 1-4 hours after administration of drug, and
- It was observed daily thereafter for a total of 14 days, except when they needed to be removed from the study and killed humanely for animal welfare reasons or are found dead.

a. Mortality

Animals will be observed intensively at 0.5, 2.0, 4.0, 6.0, 12.0, 24.0 and 48.0 hours following drug administration on day 1 of the experiment and daily twice thereafter for 14 days.

b. Body weight

Body weights will be recorded at day: -1, day 1, 2, 7 and 14 of the study

c. Cage-side observation

These include changes in skin and fur, eyes and mucous membranes and also respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behaviour patterns. Attention should be directed to observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma.

d. Gross necropsy

All animals (including those which die during the test period are removed from the study) will be subjected to gross necropsy. Gross necropsy includes examination of the external surface of the body, all orifices, cranial, thoracic and abdominal cavities and their contents, brain, eye, thymus, lungs, heart, spleen, liver, kidneys, adrenals, testes and uterus of all animals

Histopathology

Microscopic examination will be carried out in organs to show the evidence of any toxicity in gross pathology.

Data and reporting

All the data were summarised in tabular form showing the animals used, number of animals displaying signs of toxicity, the number animals found dead during the test or killed for humane reasons, a description and the time course of toxic effects and reversibility, and necroscopic findings.

Test substance and Vehicle

In order to ensure the uniformity in drug distribution in the medium the suspension was made by mixing TVC with 2% CMC solution and it was found suitable for dose accuracy.

Justification for choice of vehicle

The vehicle selected as per the standard guideline was pharmacologically inert and easy to employ for new drug development and evaluation technique.

4.3.2. 28 Days repeated dose oral toxicity study of "*Thathu Viruthi Chooranam"* on rats – (OECD-407 guidelines) [60]

Justification for Dose Selection

The results of acute toxicity studies in Wistar albino rats indicated that TVC was non-toxic and no behavioral changes was observed up to the dose level of 2000 mg/kg body weight. On the basis of body surface area ratio between rat and human, the doses selected for the study were100mg/kg, 200 mg/kg and 400 mg/kg body weight. The oral route was selected for use because oral route was considered to be a proposed therapeutic route.

Preparation and administration of dose

TVC at three doses respectively was suspended in 2 ml of 2% CMC in distilled water. It was administered to animals at the dose levels of 100, 200 and 400 mg/kg. The test substance suspensions were freshly prepared every day for 28 days. The control animals were administered vehicle only. Administration was by oral (gavage), once daily for 28 consecutive days. (IAEC No: IAEC/XLVIII/10/CLBMCP/2016)

Methodology

Randomization, Numbering and Grouping of Animals

Ten rats (Five Male and Five Female) were in each group randomly divided into four groups for dosing up to 28 days. Animals were allowed acclimatization period of 7 days to laboratory conditions prior to the initiation of treatment. Each

animal was fur marked with picric acid. The females were nulliparous and non-pregnant.

Observations

Experimental animals were kept under observation throughout the course of study for the following

Body Weight

Weight of each rat was recorded on day 0, at weekly intervals throughout the course of study and at termination to calculate relative organ weights. From the data, group mean body weights and percent body weight gain were calculated.

Clinical signs

All animals were observed daily for clinical signs. The time of onset, intensity and duration of these symptoms, if any, were recorded.

Mortality

All animals were observed twice daily for mortality during entire course of study.

Functional Observations

At the end of the 4^{th} week exposure, 'sensory reactivity' to graded stimuli of different types (auditory, visual and proprioceptive stimuli), 'motor reactivity' and 'grip strength' were assessed.

Laboratory Investigations

Following laboratory investigations were carried out on day 29 in animal's fasted over-night. Blood samples were collected from orbital sinus using sodium heparin (200IU/ml) for Blood chemistry and potassium EDTA (1.5 mg/ml) for Haematology as anticoagulant. Blood samples were centrifuged at 3000 rpm for 10 minutes. On 28th day of the experiment, 24 hours urine samples were collected by placing the animals in the metabolic cage with free access to tap water but no feed was given.

The urine was free from faecal contamination. Toluene was used as a preservative while collecting the sample. The sediments present in the urine were removed by centrifugation and the collected urine was used for biochemical estimations. On 29th day, the animals were fasted for approximately 18 hours, then slightly anesthetized with ether and blood samples were collected from the retro-orbital plexus into two tubes: one with EDTA for immediate analysis of haematological parameters, the other without any anticoagulant and was centrifuged at 4000 rpm at 4 °C for 10 minutes to obtain the serum. Serum was stored at 20 °C until analyzed for biochemical parameters.

Haematological Investigations

Blood samples of control and experimental rats was analyzed for hemoglobin content, total Red Blood Corpuscles (RBC), White Blood Corpuscles (WBC) Count and Packed Cell Volume (PCV).

Biochemical Investigations

Serum was used for the estimation of biochemical parameters. Samples of control and experimental rats were analyzed for protein, bilirubin, urea, BUN, creatinine, triglyceride, cholesterol and glucose levels was carried using standard methods. Activities of glutamate oxaloacetate transaminase / Aspartate aminotransferase (GOT/AST), glutamate pyruvate transaminase / Alanine amino transferase (GPT/ALT) and alkaline phosphatase were estimated as per the colorimetric procedure.

Urine analysis

Urine samples were collected on end of treatment for estimation of normal parameters. The estimations were performed using appropriate methodology.

Necropsy

All the animals were sacrificed on day 29. Necropsy of all animals was carried out and the weights of the organs including liver, kidneys, spleen, brain, heart, and lungs were recorded. The relative organ weight of each animal was then calculated as follows;

$$\text{Relative organ weight} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of animal on sacrifice day (g)}} \times 100$$

Histopathology

Histopathological investigation of the vital organs was done. The organ pieces (3-5µm thick) of the highest dose level of 400 mg/kg were preserved and were fixed in 10% formalin for 24 hours and washed in running water for 24 hours. Samples were dehydrated in an auto technique and then cleared in benzene to remove absolute alcohol. Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50°C and then in a cubical block of paraffin made by the "L" moulds. It was followed by microtome and the slides were stained with Haematoxylin-eosin. The organs included heart, kidneys, liver, ovary, pancreas, brain, spleen and stomach, of the animals were preserved they were subjected to Histopathological examination.

Statistical analysis

Findings such as clinical signs of intoxication, body weight changes, food consumption, haematology and blood chemistry were subjected to One-way ANOVA followed by Dunnet's multi comparision test using a computer software programme GRAPH PAD INSTAT-3 version.

4.4. PHARMACOLOGICAL STUDY

4.4.1. Evaluation of Spermatogenic activity of *Thathu Viruthi Chooranam* in ethanol induced Wistar male rats

Aim

To evaluate the spermatogenic activity of *Thathu Viruthi Chooranam* (TVC) *in Wistar* albino rats by Ethanol induced method[61].

Procurement and rearing of experimental animal

Adult male Wistar rats weighing 180-210 gms were used for this study. The inbred animals were procured from the animal house of Kings institute, Chennai and the study was conducted at C.L, Baid Metha College Of Nursing, Chennai, India.

They were housed six per cage under standard laboratory conditions at a room temperature at 22 ± 2^0 C. The animals were subjected under standard photoperiodic condition of 12:12 hrs light: dark cycle. The animals were fed with standard rodent pellet and water *ad libitum*. Animals were acclimatized to laboratory conditions one week prior to initiation of experiments. The protocol for experimentation was approved by Institutional Animal Ethics Committee.

Experimental design

Sample Size: 24 albino rats , Experiment Duration: 60 days

Animal grouping and interventions

The animals were randomly selected and divided into four groups (I, II, III and IV) of six rats (n=6) each. Individual identification of the animal was made by marking. Group I animals served as control and received only 1ml milk, p.o. for 60 days. *Group II* served as negative control (Alcohol induced), received 0.5 ml of 25% ethanol /kg /BW /day for 60 days. Experimental groups splits into group III and IV served as the treated groups and received TVC which was grounded in mortar-pestle with milk. *Group III* was treated with 25% of 0.5 ml ethanol /kg/BW/day along with 100mg/kg of TVC orally once for 60 days. . *Group* IV was treated with 25%of 0.5 ml ethanol /kg/BW/day along with 200mg/kg of TVC orally once for 60 days Administration was done once a day by oral gavage in the morning.

Table No.6. Grouping of animals for the evaluation of Spermatogenesis activity of TVC

Groups	Intervention	No of Rats
Group I - Normal Control	1 ml of Milk	6
Group II - Negative Control	25% of ethanol (0.5 ml /kg/day)	6
Group III - Treatment group	TVC (100mg / kg / day) + 25% of ethanol (0.5 ml /kg/b.w/day)	6
Group IV - Treatment group	TVC (200mg / kg b.w / day) + 25% of ethanol (0.5 ml /kg/b.w/day)	6

Sampling, Sacrifice and Surgical procedure

Twenty-four (24) hours after the 60th day of treatment, following over-night fasting (12 hrs), the animals were sacrificed with i.p. (intraperitonial) injection of thiopentone. The abdominal cavity was opened up through a midline abdominal incision to expose the genital organs. Testes, Prostate, seminal vesicle and epididymis were excised, trimmed of all fat and other tissues, mopped with tissue paper and then weighed. Left testicles were collected to monitor the spermatozoal characteristics and Right testicles to conduct testicular and epididymal histopathology .The section was studied microscopically for changes in histo architecture or morphology. The left caudal epididymis were transferred into sterile bottles containing 2 ml of normal saline for semen analysis. Semen samples from caudal epididymis (left) were subjected to parameters such as count, motility, viability and abnormality. Counting was performed using a haemocytometer and light microscope with 100X.

Enumeration of sperm parameters

Semen analysis

Examinaion of sperm count, sperm motility, viability and spermatozoal abnormalities was carried out by making small cuts in the area of the cauda epididymis close to the vas deferens and apply gentle pressure to exude epididymal contents

Sperm Count

The sample was drawn into WBC pipette and diluted to the ratio of 1:100 with the modified Krebs Ringer-bicarbonate buffer containing 0.05% collagenase (pH 7.4) followed by this 1:1000 dilution was performed with 1.8% NaCl and 2% formalin). The sperm suspension was placed in the haemocytometer with improved double Neubauer ruling was used for the counting of spermatozoa. Counts for 2-4 haemocytometer chambers were averaged. The sperm suspension was evaluated for sperm count.

Total number of sperm cells in all the four chamber = X

X multiplied by 10,000 to obtain the number of cells (Y) per ml of diluted sample

Y multiplied by 100 (the dilution factor) to obtain (Z) sperm cells per ml of original semen sample.

Sperm Motility[62]

The sample was mixed with 20mm HEPES (4-(2-hydroxyethyl)-1-piperazine ethane sulfonic acid contains L-Glutamine with 5% BSA (*Bovine serum albumin*) . Final sample suspension mixed with formalin and used to assess motility. Bright field microscope magnification 100x.

$$\text{Motility (\%)} = \frac{\text{Number of motile spermatozoa}}{\text{Total number of spermatozoa (Motile + Immotile)}} \times 100$$

Percentage viability[63]

Viability was assessed by eosin Y staining (5% in saline). Forty micro litre samples of the freshly sperm suspension were placed on a glass slide, mixed with 10 µL eosin and observed under a light microscope (x400 magnification). Live sperms remained unstained following staining; whereas, those that showed any pink or red colouration were classified as dead. At least 200 sperm were counted from each sample in ten fields of vision randomly, and the percentage of live sperms was recorded

$$\text{Percentage of sperm viability} = \frac{\text{Total Viable cells (Unstained)}}{\text{Total cells (Viable + Dead)}} \times 100$$

Sperm morphology[64]

Staining

A suggested method for staining uses 1ml of sperm suspension which was transferred to a test tube. Two drops of 1% eosin Y were added to the test tube and mixed by gentle agitation. The above mixture was incubated at room temperature for approximately 45-60 minutes to allow for staining.

Slide preparation

Slides should be cleaned with detergent, washed in water followed by alcohol and dried before use. One to two drops of the stained sperm suspension were placed approximately 1cm from the frosted end of a pre-cleaned microscope slide lying on a flat surface. A second slide was held in the right hand with the Z slides's long edge gently touching across the width of the sperm slide and pulled across to produce a sperm smear. After drying the smears were fixed with formalin.

Characterization of normal and abnormal sperm

Abnormality in sperms were calculated based on the following parameter like curved tail, Tail less head, Headless tail, looped tail and coiled tail etc. Normal sperm were calculated based on the appearance and absence of above mentioned parameters.

$$\text{Percentage of normal sperm} = \frac{\text{No of Normal sperm}}{\text{Total number of sperms in the filed}} \times 100$$

$$\text{Percentage of normal sperm} = \frac{\text{No of abnormal sperm}}{\text{Total number of sperms in the filed}} \times 100$$

Procedure for histopathology[65]

The rats from each group were anethetized by drug with out any injury after lower pelvic region. The collected samples were washed with normal saline and fixed in 10% neutral formalin for 48 hrs for further histological observation. Paraffin section were taken at 5 μm thickness processed in alcohol-xylene series and was stained with Haematoxylin-eosin dye. The sections were examined microscopically for histopathological changes. The magnification for low power was carried out at 10 X and for high power at 45 X.

Statistical analysis

Data collected in the study were expressed as the mean ± SEM and statistical analysis was carried out using Dunnett test. P value less than 0.05 was considered to be statistically significant. All data were summarized in tabular form Table (18-20).

4.4.2. Evaluation of Aphrodisiac Activity of *Thathu Viruthi Chooranam* in ethanol treated male rats

Aim

To evaluate the aphrodisiac potential of *Thathu Viruthi Chooranam* in ethanol treated Wistar albino male rats.

Animal procurement and maintenance

Wistar Albino rats of either sex, weighing 150 g to 200 g were purchased from King Institute of Preventive medicine Animal House, Chennai, India and they were acclimatized in Animal house of C.L Baid metha college of pharmacy, Gerugambakkam, Chennai, India at 21-23ºC. Animal ethical guidelines of CPCSEA, Ministry of Animal Husbandry and Welfare, Govt. of India were strictly followed for the care and maintenance of procured animals. The animals were fed on standard rodent pellet and RO water was provided *ad libitum.* Animals were acclimatized to laboratory conditions one week prior to initiation of experiments. The protocol for experimentation was approved by Institutional Animal Ethics Committee (IAEC).

Experimental Details

The sexually active male rats were chosen separately and divided into 6 groups; each group consisting of 6 animals. The animals in the divided groups received the treatment orally.

The sexual behavior of the experimental rats was observed in a dark light in specially designed cages that have glasses on all the sides and measuring 50×30×30cm. The male experimental rat was first placed in the cage and then two female rats in estrous phase were introduced. An initial period of 15 minutes was considered as acclimatization period. After 15 minutes, the extract or the drug was introduced and the activity of male rat in each group was recorded individually for 60

minutes, after 30 minutes of drug administration. To determine the aphrodisiac activity of the extracts, several parameters were observed. These include measuring and observing the mount frequency, mount latency, intromission frequency, intromission latency, genital grooming and anogenital sniffing[66-69].

Definitions of individual parameters observed

Mount frequency

Mount Frequency is corresponded to the number of mounts without intromission from the time of introduction of the female until ejaculation.

Intromission frequency

Intromission is the introduction of one organ or parts into another. e.g. the penis into the vagina. Intromission Frequency is therefore defined as the number of intromissions from the time of introduction of the female until ejaculation.

Mount latency

Mount Latency is defined as the time interval between the introduction of the female and the first mount by the male.

Intromission latency

Intromission Latency is the time between the introduction of the female and the first intromission. This is usually characterized by pelvic thrusting, and springing dismounts.

Ejaculatory latency

Ejaculatory Latency is defined as the time from the first intromission to the first ejaculation.

Post-ejaculatory interval

The post-ejaculatory interval is the time between ejaculation and the forth coming non ejaculatory intromission. The test is negative if the latency of intromission and ejaculation is greater than 20 minutes.

Sperm count (no of sperm x 10^6)

After treatment, the sperm count was carried out by using Haemocytometer (Mukherjee and Kanai, 1988). Haemocytometer is generally used for RBC as well as WBC count. It is provided with the pipettes for the dilution of the blood samples and Neaubaur's slide with special type of ruling. The counting was done in the ruled squares on the slide. The epididymis was removed and placed in a pre-chilled petri-plate. 2 ml. of 0.9% saline was added to it and the cauda epididymis was gently minced with the help of sharp razor. This sample was used for the sperm count. The sample was pipetted out with the help of pipette provided in the Haemocytometer. A clean and dry cover slip was kept on the Neaubaur's ruling. The ruling was loaded with the sample by touching the tip of the pipette to the slide.

Histopathological Analysis

At the end of 28^{th} day testis were isolated for histopathological examination and fixed in 10 % formal saline (10 parts of formaldehyde and 30 parts of normal saline). Tissues were processed and embedded in paraffin wax. Sections were cut at 5 micron thickness and stained with Haematoxylin and Eosin. Light microscopic examination of the sections was then carried out and micrographs produced using Vanox-T Olympus photographing microscope. The histopathological examinations were reviewed by the pathologist.

Statistical analysis

Data collected in the study were expressed as the mean ± SEM and statistical analysis was carried out using Dunnett test. P value less than 0.05 was considered to be statistically significant. All data were summarized in tabular form Table.

4.4.3. Evaluation of antioxidant activity of *Thathu Viruthi Chooranam* through DPPH (2, 2-Diphenyl 1-2 picrylhydrazyl) Assay

The antioxidant activity of *Thathu Viruthi Chooranam* was determined using the 2, 2-diphenyl-1 picrylhydrazyl (DPPH) free radical scavenging assay[70].100µl of *Thathu Viruthi Chooranam* extract was mixed with 2.7ml of methanol and then 200µl of 0.1 % methanolic DPPH was added. The suspension was incubated for 30 minutes in dark condition. Initially, absorption of blank sample containing the same amount of

methanol and DPPH solution was prepared and measured as a control subsequently, at every 5 min interval, the absorption maximum of the solutions were measured using a UV double beam spectra scan (Chemito, India) at 517nm. The antioxidant activity of the sample was compared with known synthetic standard of 0.16% Butylated Hydroxy Toluene (BHT). The experiment was carried out in triplicates[71,72].

Free radical scavenging activity was calculated by the following formula:

$$\% \text{ Inhibition} = \frac{(\text{Abs of Control} - \text{Abs of Test})}{\text{Abs of Control}} \times 100$$

PREPARATION OF *THATHU VIRUTHI CHOORANAM*

Fig No.1.5. Juices of *Curculigo orchioides* (Whole plant)

Pounding

Grinding

Fig No.2. Image of *Thathu Viruthi Chooranam*

RESULTS AND DISCUSSION

5. RESULTS AND DISCUSSION

The classical medicine *Thathu Viruthi Chooranam* for *Aan maladu* (Male infertility) which was subjected to various evaluations to standardize the quality and efficacy of the medicine through scientific way, and prove the genuinity of the classical literature mentioned in our Siddha system of medicine. Literary collection, physicochemical analysis, phytochemical screening, Bio-chemical analysis toxicity studies, and pharmacological activities are done to prove the Aphrodisiac, Spermatogenic, Antioxidant activity. The literary review of the drug from various, text books and journals gave great hope about its spermatogenic activity. These studies insist that the strong value of the results. They are discussed one by one below.

Standardization of the drug

Standardization of the drug was very important to achieve the therapeutic efficacy, potency of the trail drug by evaluating the drug by doing several analyses. The physical character and the physicochemical analysis have been done and the results are tabulated. The Bio chemical analysis of the trail drug was done and tabulated. Toxicological study results and pharmacological study results also derived, and tabulated below. The microbial load of the trail drug was done and the results were tabulated separately. Thus these studies bring out the complete justification about the trail drug *Thathi Viruthi Chooranam* for its Spermatogenic, Aphrodisiac, Antioxidant activity.

Organoleptic character

Table No.7. Organoleptic characteristics properties of TVC

Colour	Brown
Odour	Pleasant
Taste	Sweet
Texture	Fine powder
Particle size	Completely pass through sieve no 88

Physicochemical analysis

Table No.8. Physicochemical analysis properties of TVC

S.NO	Parameter		Result
1	pH		5.58
2	Ash (%)		7.985
3	Acid Insoluble ash (%)		1.820
4	Water soluble ash		1.685
5	Loss on drying(at 105^0 C)		7.66
6	Alcohol soluble extractive		28.60
7.	Solubility		
	I	Distilled water	Soluble
	II	Benzene	Soluble
	III	Chloroform	Soluble
	IV	Carbon tetra chloride	Soluble
	V	Xylene	Soluble
	VI	Petroleum ether	Soluble

Interpretation

A value characteristic of an aqueous solution is its pH value, which represents conventionally its acidity or alkalinity[73]. The pH scale represents the relative concentration of hydrogen ions in a solution. The concentration of Hydrogen ions is commonly expressed in terms of the pH scale. Low pH corresponds to high Hydrogen ion concentration and vice versa. In the trial drug *Thathu Viruthi Chooranam* the pH

is 5.58 which represents the concentration of Hydrogen ions is more when it is in the form of a solution[74].

One Research study found that acids had higher oral bioavailability and was likely to be the result of better solubility and lower clearance[75]. So, the result concludes that the oral bioavailability of the drug *Thathu Viruthi Chooranam* is very high.

Ash is the inorganic residue left after ignition at 650-700°C. The ash content is an approximate measure of the mineral content and other inorganic matter in biomass. The ash content is a measure of the total amount of minerals present within a food, whereas the mineral content is a measure of the amount of specific inorganic components present within a food, such as Ca, Na, K and Cl.

The quality of drugs depends on the concentration and type of minerals they contain, including their taste, appearance, texture and stability. Ash is one of the components in the proximate analysis of biological materials, consisting mainly of salty, inorganic constituents. It includes metallic salts which are important for processes requiring ions such as Na^+ (Sodium), K^+ (Potassium), and Ca^{2+} (Calcium). The ash value of *Thathu Viruthi Chooranam* indicates the presence of minerals such as Sodium, Potassium and Calcium. The acid insoluble value of the drug *Thathu Viruthi Chooranam* is 1.82 %.

Solubility is the basic requirement for the absorption of the drug from GIT. Here the water soluble nature of *Thathu Viruthi Chooranam* is 31.7 %. This nature might be helpful for the better absorption. To determine the moisture content of a sample, although occasionally it may refer to the loss of any volatile matter from the sample. Here the loss of drying value of the trial drug *Thathu Viruthi Chooranam* is 7.66 % @ 105°C (%).

Phytochemical analysis

Table No.9. Results of phytochemical findings of TVC

Phytochemicals	Test used	Chloroform	Methanol
Alkaloids	Dragendroff	-	+
Flavonoids	Shinado	+	+

Phytochemicals	Test used	Chloroform	Methanol
Glycosides	Legal's test	+	+
Saponins	Foam test	-	-
Tannins	Ferric chloride	+	-
Phytosterol	Liberman	-	-
Triterpenoids	Noller's test	+	-

Interpretation

On Phyto chemical analysis the drug *Thathu Viruthi Chooranam* possess the presence of some major phytochemicals such as alkaloids, flavonoids, glycosides, tannins, triterpenoids in choloroform and methanolic extract. These phytochemicals have high range of therapeutic use. The alkaloids have more medicinal property. Some alkaloids are identified for the beneficial use for the treatment of infertility for example, a new ergot alkaloid (2-Br-alpha-ergocryptine)[76].

Alkaloids and Triterpenoids

Alkaloids and Triterpenoids were confirmed and are important to cure the chronic diseases and nervine disorders. The availability of Flavonoids in the trial drug clearly indicates the drug's potency against the degenerative changes and its anti-oxidant property.

Flavonoids

Flavonoids have vaso relaxant properties, which may caused by increase in no production in vascular bed and a decrease in its destruction.

Tannins

The availability of Tannins in the trial drug indicates its neuro protective property.

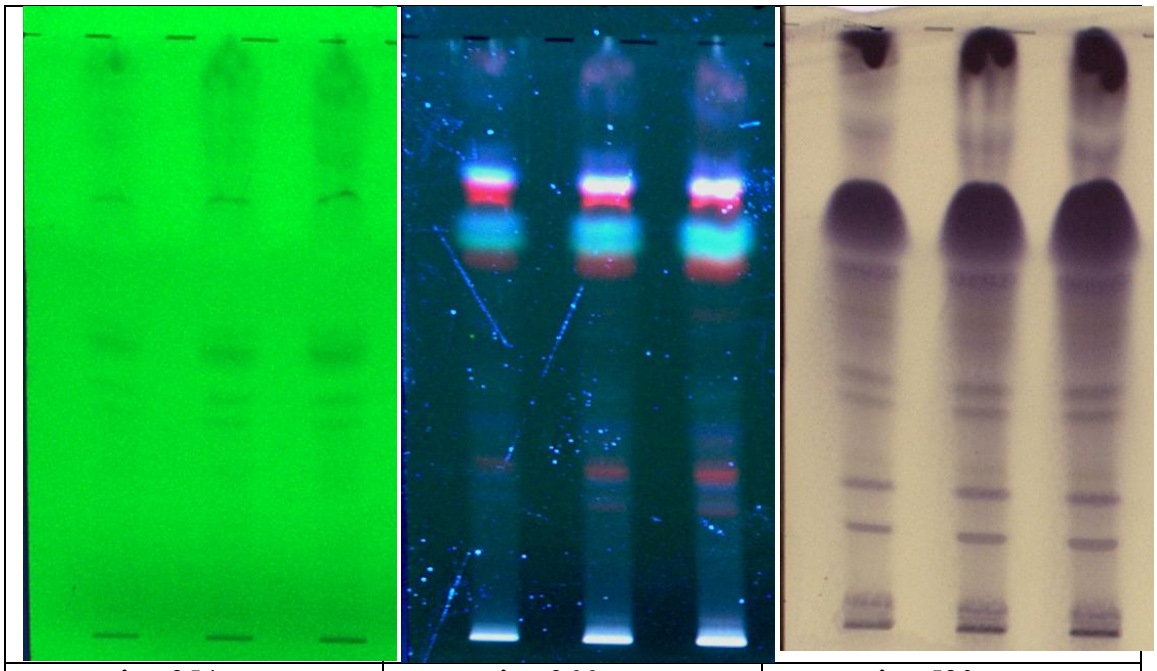
By the available phytochemicals, the trial drug has the therapeutic potency of vaso dilatation, enhancing libido and neuro protective in the erectile dysfunction.

TLC/ HPTLC analysis of chloroform extract

TLC Photo documentation : Thathu Viruthi Chooranam

Stationary Phase - Silica Gel 60 F_{254}

Mobile Phase – Toluene: Ethyl acetate: Formic Acid (9:2:0.2 v/v/v)



λ = 254 nm	λ = 366 nm	λ = 520 nm

Table No.10. Rf values for the Chloroform extract

R*f*	Colour	R*f*	Colour	R*f*	Colour
0.36	Light	0.22	Red	0.03	Violet
0.40	Light	0.28	Red	0.14	Violet
0.47	Dark	0.39	Green	0.22	Violet
0.52	Light	0.62	Red	0.36	Violet
0.73	Dark	0.67	Green	0.39	Violet
		0.70	Blue	0.50	Yellow
		0.74	Red	0.58	Violet
		0.76	White	0.67	Violet

- Under UV 254nm, it shows 5 major spots at Rf 0.36, 0.40, 0.47, 0.52, 0.73 major compounds are found.
- Under UV 366nm, it shows 8 major spots at Rf 0.22, 0.28, 0.39, 0.62, 0.67, 0.70, 0.74, 0.76 major compounds are found.

- Under UV 366nm, it shows 8 major spots at Rf 0.03, 0.14, 0.22, 0.36, 0.39, 0.50, 0.58, 0.67 major compounds are found.

3D Chromatogram of 254 nm

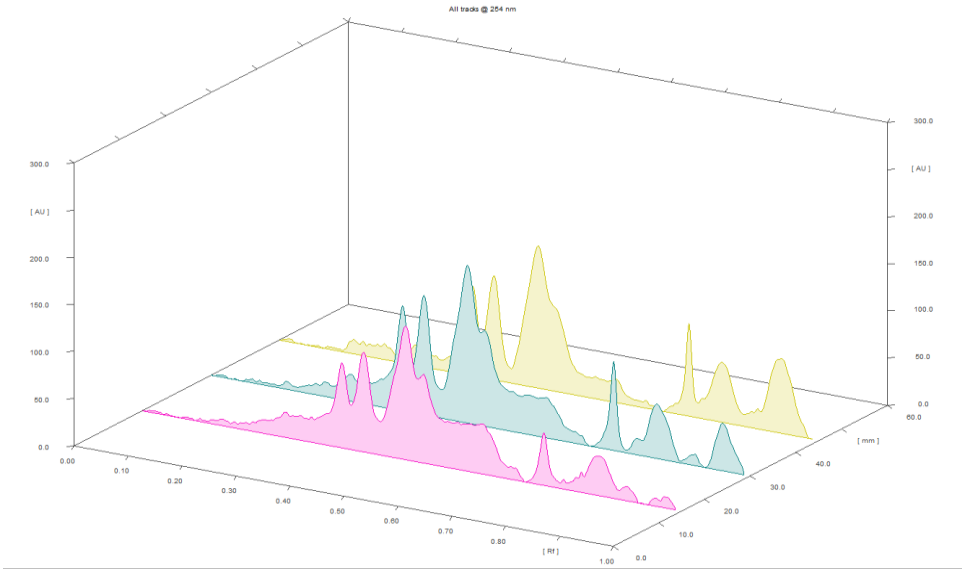


Fig No.7. Image of 3D Chromatogram

HPTLC Chromatogram of Chloroform extract scanning at 254 nm

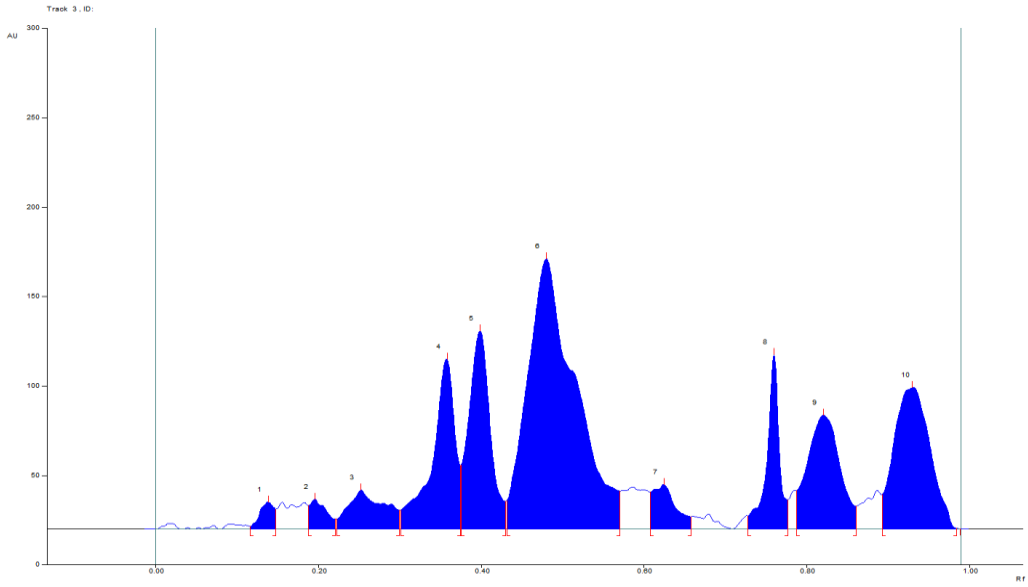


Fig No.8. Image of HPTLC Chromatogram

Table No.11. Peak Table@254 nm

Track 3, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.12 Rf	1.3 AU	0.14 Rf	15.3 AU	2.26 %	0.15 Rf	11.1 AU	238.0 AU	1.13 %
2	0.19 Rf	13.0 AU	0.20 Rf	16.6 AU	2.46 %	0.22 Rf	5.5 AU	302.9 AU	1.43 %
3	0.22 Rf	5.6 AU	0.25 Rf	21.8 AU	3.23 %	0.30 Rf	10.5 AU	835.6 AU	3.95 %
4	0.30 Rf	10.6 AU	0.36 Rf	95.0 AU	14.05 %	0.37 Rf	35.6 AU	2298.7 AU	10.87 %
5	0.38 Rf	35.8 AU	0.40 Rf	110.9 AU	16.40 %	0.43 Rf	15.5 AU	2536.1 AU	11.99 %
6	0.43 Rf	15.8 AU	0.48 Rf	151.1 AU	22.34 %	0.57 Rf	21.1 AU	7720.1 AU	36.51 %
7	0.61 Rf	20.6 AU	0.63 Rf	24.9 AU	3.68 %	0.66 Rf	6.9 AU	632.8 AU	2.99 %
8	0.73 Rf	7.3 AU	0.76 Rf	97.8 AU	14.46 %	0.78 Rf	16.3 AU	1301.2 AU	6.15 %
9	0.79 Rf	21.4 AU	0.82 Rf	63.7 AU	9.41 %	0.86 Rf	12.7 AU	2223.0 AU	10.51 %
10	0.89 Rf	19.2 AU	0.93 Rf	79.2 AU	11.71 %	0.99 Rf	0.4 AU	3056.7 AU	14.46 %

Interpretation

- The quantitative analysis of compounds present in the TVC has been performed by HPTLC. The method may be applied to identify the TVC from other manufacturing process. It provides the identification of constituents, determination of impurities and quantitative determination of active substance present in TVC[77].
- The Rf value of the TVC supports the better standardization of the drug.
- The present study revealed that TVC showed best results in Toluene: Ethyl Acetate (9:2) Solvent system. After scanning and visualizing the plates in absorbance mode at 254nm, 366nm and 520nm. Best results were shown at visible light range.
- TLC plate showed different colour phytoconstituents of chloroform extract of TVC. The bands revealed presence of seven violets, four reds, two greens, one blue, one white and one yellow, bands showing the presence of alkaloids, glycosides, phenols, triterpenes, flavonoids and quinines.

The results from HPTLC finger print scanned for chloroform extract of TVC. There are thirteen polyvalent phytoconstituents and corresponding ascending order of

Rf values start from 0.12 to 0.89 in which highest concentration of the phytoconstituents was found to be 22.34% and 16.40% with its corresponding Rf value found to be 0.12 and 0.89 respectively.

Bio chemical analysis

Determination of basic radicals

Table No.12. Results of basic radicals of TVC

S.NO	Parameter	Observation	Result
1	Test for Calcium	White colour precipitate	+ve
2	Test For Magnesium	White colour precipitate	+ve
3	Test For Zinc	White colour precipitate	+ve

Interpretation

The drug shows the presence of Calcium, Magnesium and Zinc in basic radicals determination.

Calcium

Calcium ions are playing important role in the squeezing and relaxing of muscle, it is one of the micro nutrient which help to release hormones and other chemicals. It is sending and receiving nerve signals also. So Ca ions help to promote the nerve signals and release hormones, regulates squeezing and relaxing of muscle in erectile dysfunction. Also it keeps heart rate normally.

Magnesium

Magnesium ions have a role in oxidation of fatty acids, activation of amino acids and neurotransmission. It is the fourth most abundant mineral in the human body and essential to good health.

Zinc

Zinc is the important substance for vaso dilatation. So it may increase the blood flow in the penis and increases the Libido, and can helpful in erectile dysfunction.

Following above description, the major ions are performing an important role in the erectile disorders and promotes erection. They decrease the symptoms and signs.

Determination of Acid Radicals

Table No.13. Results of the estimation of acid radicals of TVC

S.NO	Parameter	Observation	Result
1	Test for Sulphate	Formation of white precipitate	+ ve
2	Test for Chloride	Formation of white precipitate	+ ve

Interpretation

The drug exhibits the presence of sulphide and chloride in acid radicals estimation.

Sulphate

- Increases sperm quality and quantity
- Important in sperm function
- Provides antioxidant protection for sperm
- Increases libido

Chloride

- Help to promote the nerve signals and release hormones
- Relaxing of muscle in erectile dysfunction

Microbial load

Availability of bacterial and fungal load in *Thathu Viruthi Chooranam*

Table No.14. Bacterial and Fungal dilutions

MICROBES	DILUTION	RESULT
BACTERIA	10^{-4}	8
BACTERIA	10^{-6}	6
FUNGI	10^{-2}	5
FUNGI	10^{-3}	3

Interpretation

- The availability of bacterial load in the TVC has been performed by Agar plate technique.
- As TVC is made from plant material it is more prone to contamination. The contamination of herbal drugs by microorganism not only cause bio deterioration but also reduces the efficacy of drugs.
- The toxin producesd by microbes makes herbal drugs unfit for human consumption because the contaminated drug may develop unwanted disease instead of disease being cured.
- The contamination of TVC has been examined by bacterial and fungal load.
- Total bacterial load in 10^{-4} dilution is 8 and 10^{-6} dilution 6.
- Total fungal load in 10^{-2} dilution is 5 and 10^{-3} dilution is 3.

This result shows the presence of bacterial and fungal load in the trial drug (TVC). The load of bacteria and fungi are within the limits of WHO norms.

Instrumental analysis

FTIR Spectrum analysis

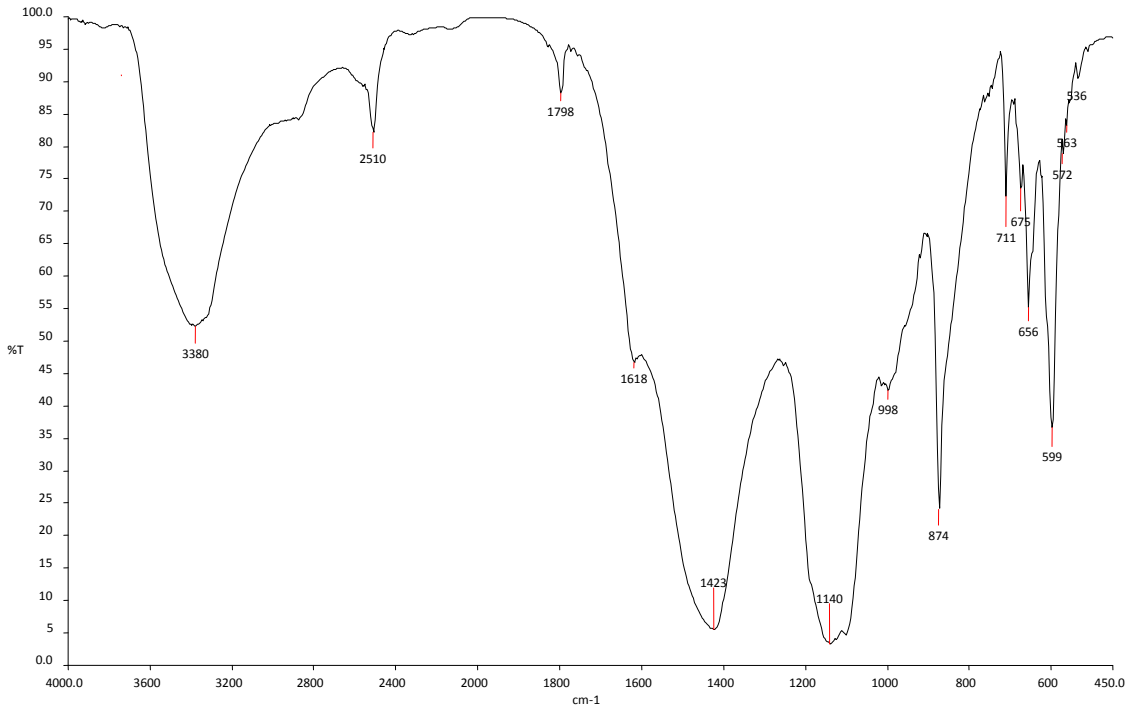


Fig No. 9. Image of FTIR Spectrum

Table No.15. FTIR data interpretation of TVC

Wave number (cm-1)	Vibrational modes of TVC in IR region	Functional groups
3380	O-H stretch, H-bonded	Alcohols , Phenols
1618	N-H bend	1 Amine
1423	C-C stretch	Aromatics
1140	C-N stretch	Aliphatic amines
998	C-H bend	Alkenes
874	N-H wag	1,2 Amines
711	C-H “oop”, =C-H bend	Aromatics, Alkenes
675	=C-H bend, C-H “oop”	Alkenes, Aromatics
656	C-Cl stretch, C-Br stretch	Alkyl halides
599	C-Cl stretch	Alkyl halides

Wave number (cm-1)	Vibrational modes of TVC in IR region	Functional groups
572	C-Br stretch	Alkyl halides
563	C-Cl stretch	Alkyl halides
536	C-Br stretch	Alkyl halides

Interpretation

In FT-IR Spectra analysis, this sample *Thathu Viruthi Chooranam* exhibits the peak value at 3380, 1618, 1423, 1140, 998, 874, 711, 675, 656, 599, 572, 563, 536 having O-H stretch, H-bonded, N-H stretch, N-H bend, C-N stretch, C-H bend, N-H wag, C-H "oop", C-Cl stretch, C-Br stretch. This indicates the presence of some organic functional groups such as alcohols, phenols, alkanes, 1,2 amines, aromatics, alkyl halides, amides, aliphatic amines. The OH group has higher potential towards inhibitory activity against microorganisms. Sometimes the presence of Phenols in medicinal plants possess highly Anti-Oxidant property which enhances the drug effect against the disease. For example, the phenolic compound of Hypericum perforatum[78].

Scanning electron microscopy (SEM)

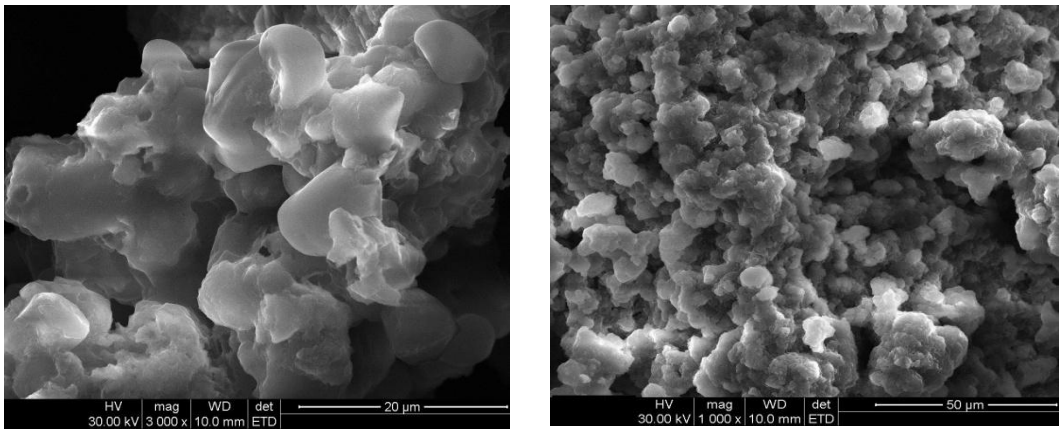


Fig No.10. SEM images of TVC

Interpretation

In the above SEM studies of the drug *Thathu Viruthi Chooranam* showed objects of sizes ranging from 3µm to 5µm. The surface of the sample grains is uniformly arranged in agglomerates. These micro sized particles of this *Chooranam*

helps better absorption. Thus can conclude that the therapeutic efficacy of the drug always good in nature.

XRD – X ray Diffraction Studies

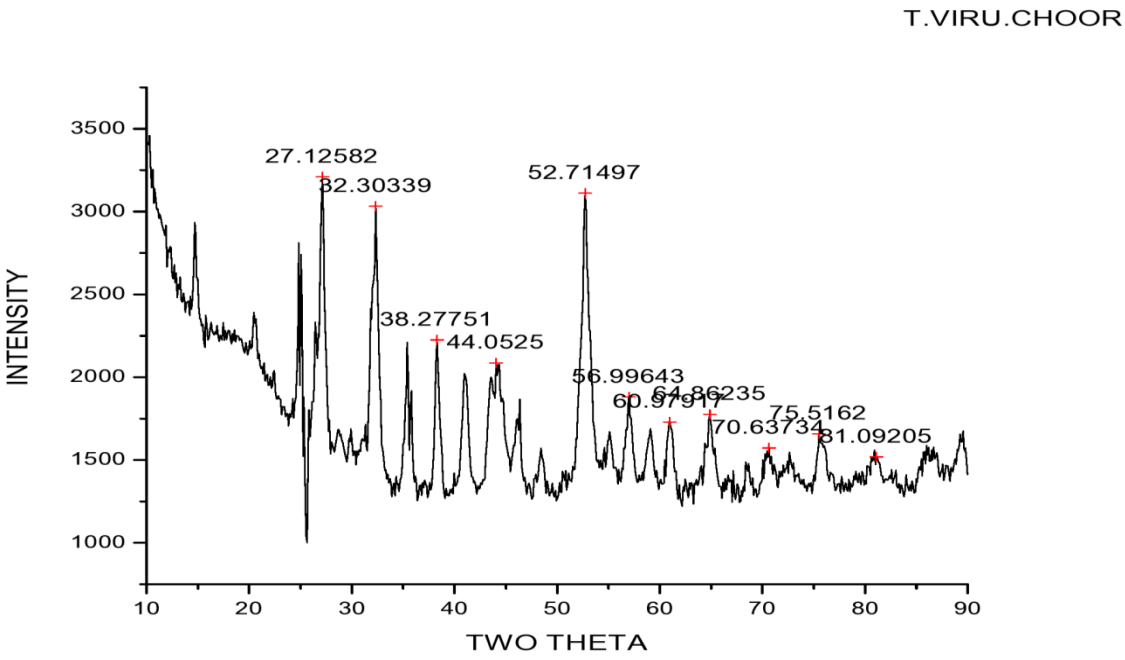


Fig No.11. XRD image of TVC

Interpretation

The structure, the size and shape of the particles are highly dependent on the route of synthesis and high lights the efficacy of the drug. The micro particles may enhance bio absorption of the drug.

The major diffraction peaks are identified after XRD analysis TVC concluded that range is 26-54nm associated with organic molecules probably plays an important role in making it biocompatible and nontoxic at therapeutic doses. Other elements present in TVC act as additional supplement and possibly helps in increase the efficacy of the formulation.

ICP-OES

Table No.16. ICP-OES findings of TVC

S. No	Elements	Detected levels(mg/L)
1.	Aluminium	BDL
2.	Arsenic	BDL
3.	Calcium	12.180
4.	Cadmium	BDL
5.	Copper	BDL
6.	Iron	15.340
7.	Mercury	BDL
8.	Potassium	53.891
9.	Megnesium	01.354
10.	Sodium	04.300
11.	Nickel	BDL
12.	Lead	BDL
13.	Phosphorus	86.300
14.	Sulfur	01.001
15.	Zinc	03.250

Interpretation

- The presence of Ca (12.180 mg/l), K (53.891 mg/l), Fe (15.340mg/l), Mg (01.354mg/l), Na (04.300mg/l), P (86.300mg/l), S (01.001mg/l), Zn (03.250mg/l) is physiologically important. In *Thathu Viruthi Chooranam*, the heavy metals like Al, As, Cd, Cu, Hg, Ni and Pb were below detectable level. This reveals the safety of the drug.

- From the above results, that the trial drug is safe as it contains heavy metals are observed within the permissible limits. Hence the safety of the drug *Thathu Viruthi Chooranam* is ensured.

TOXICOLOGICAL RESULTS

Acute oral toxicity study of *Thathu Viruthi Choornam* (OECD guideline – 423)

Table No.17. Dose finding experiment and its behavioral signs of acute oral toxicity

Observation done

SL	Group CONTROL	Observation	SL	Group TEST GROUP	Observation
1	Body weight	Normal	1	Body weight	Normally increased
2	Assessments of posture	Normal	2	Assessments of posture	Normal
3	Signs of Convulsion Limb paralysis	Normal	3	Signs of Convulsion Limb paralysis	Absence of sign (-)
4	Body tone	Normal	4	Body tone	Normal
5	Lacrimation	Normal	5	Lacrimation	Absence
6	Salivation	Normal	6	Salivation	Absence
7	Change in skin color	No significant color change	7	Change in skin color	No significant colour change
8	Piloerection	Normal	8	Piloerection	Normal
9	Defecation	Normal	9	Defecation	Normal
10	Sensitivity response	Normal	10	Sensitivity response	Normal
11	Locomotion	Normal	11	Locomotion	Normal
12	Muscle gripness	Normal	12	Muscle gripness	Normal
13	Rearing	Mild	13	Rearing	Mild
14	Urination	Normal	14	Urination	Normal

Table No.18. Dose finding experiment and its behavioral Sign of Toxicity for TVC

No	Dose mg/kg	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1.	Control	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2.	2000mg	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-

1.Alertness 2.Aggressiveness 3.Pile erection 4.Grooming 5.Gripping 6. Touch Response 7.Decreased Motor Activity 8.Tremors 9.Convulsions 10.Muscle Spasm 11.Catatonia 12.Muscle relaxant 13. Hypnosis 14.Analgesia 15.Lacrimation 16. Exophthalmos 17.Diarrhea 18.Writhing 19.Respiration 20.Mortality.

Table No.19. Body weight (gm/day) of Wistar albino rats group exposed to TVC

DOSE	DAYS		
	1	7	14
CONTROL	280.2±42.30	281.4 ± 64.12	282.6 ±26.18
HIGH DOSE	280.4± 21.24	281 ± 3.64	281.4 ± 2

*N.S- Not Significant,**(p > 0.01), *(p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)*

Table No.20. Water intake (ml/day) of Wistar albino rats group exposed to TVC

DOSE	DAYS		
	1	6	14
CONTROL	61 ± 1.12	62±2.22	63.9±1.14
HIGH DOSE	62.2±1.1	63±1.14	64.20±24

*N.S- Not Significant,**(p > 0.01), *(p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)*

Table No.21. Food intake (gm/day) of Wistar albino rats group exposed to TVC

DOSE	DAYS		
	1	7	14
CONTROL	56.24±2.22	56.2±7.42	58.4±3.46
HIGH DOSE	60.6±1.63	60.6±2.62	64.1±5.38

*N.S- Not Significant,**(p > 0.01), *(p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)*

Discussion

- The acute oral toxicity potentials of *Thathu Viruthi Chooranam* in female Wistar albino rats were studied effectively.
- In the sighting study, the test substance was administered in sequential manner to one animal each at 2000 mg kg-1 body weight followed by two animals at 2000 mg kg-1 body weight.
- According to OECD guidelines, for acute oral toxicity LD_{50} dose of 2000mg/kg of the drug is found to be safe.
- From the maximum tolerable dose 2000mg/kg of *Thathu Viruthi Chooranam* 1/5th or 1/10th of the dose was considered as therapeutic dose for further study.
- The treated animals were observed for mortality, untoward clinical/toxic signs, and alterations in body weight gain and necropsy findings during the study.
- The treated animals survived throughout the study period and did not reveal any treatment related major abnormal clinical signs at the test dose levels.
- Morphological characters like changes in skin, eyes, fur, nose appeared normal.
- The rats did not reveal any observable signs of central nervous system.

- The rats showed signs of alertness, grooming and touch response at the dose level of 2000mg/kg of body weight.
- The overall percentage of body weight gain in rats treated with the drug every weekly was found to be normal indicating that the test animals were in a healthy condition during the days of observation period.
- The weight gain of the animal was showed in Table. The changes in water and food intake recorded and it did not show any distinct deviations.
- On necropsy, no abnormalities were observed. In conclusion, acute oral toxicity testing of screened drug did not produce any treatment-related adverse effects.
- This indicates that the dosages administered were below toxic level and proves the safety of the drug.
- Hence the test drug *Thathu Viruthi Chooranam* is a safe herbal drug and can be used for long time administration.

Results of 28-days repeated oral toxicity in Wistar albino rats

Table No.22. Body weight of Wistar albino rats group exposed to TVC

DOSE	DAYS				
	1	7	14	21	28
CONTROL	290.2±24.22	291.4 ±14.24	291.5 ± 25.40	292.5±35.46	292.4 ±45.15
LOW DOSE	290.2 ±46.14	291.4 ±27.20	291.6± 66.74	292 ±62.18	294.8± 54.34
MID DOSE	290.4± 04.24	292.3 ±46.54	293.2± 68.16	294.4±54.26	296.4 ±64.70
HIGH DOSE	290.6± 64.94	296.6 ±50.53	310.4 ± 52.44	312 ±24.68	316 ±74.60*

***(p > 0.01),*(p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)*

Table No.23. Water intake (ml/day) of Wistar albino rats group exposed to TVC

DOSE	DAYS				
	1	6	14	21	28
CONTROL	60.2 ± 1.21	60.6±6.12	62.2±4.10	62±4.12	64.6±1.32
LOW DOSE	62.1±1.10	62.6±2.42	62.9±1.72	63.2±6.86	64.4±1.54
MID DOSE	58.1±1.26	58.3±3.21	59.1±6.41	59.4±1.72	59.4±1.82
HIGH DOSE	54.1±1.41	54.2±1.42	54.4±1.44	54.6±1.52	55.8±2.82

*N.S- Not Significant, **(p > 0.01), *(p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)*

Table No.24. Food intake (gm/day) of Wistar albino rats group exposed to TVC

DOSE	DAYS				
	2	7	23	22	28
CONTROL	36.0±4.12	36.2±3.12	37.3±2.84	37.2±1.41	38.0±2.43
LOW DOSE	38.2±1.41	38.3±1.13	38.1±1.21	39.5±1.23	39.5±1.26
MID DOSE	35.1±3.32	35.2±3.04	35.2±2.42	36.2±2.61	37.2±1.42
HIGH DOSE	37.1±1.32	37.8±1.41	39.3±2.62	39.2±1.10	40.6±3.42*

***(p > 0.01), *(p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test*

Table No.25. Haematological parameters of Wistar albino rats group exposed to TVC

Category	Control	Low dose	Mid dose	High dose
Haemoglobin (g/dl)	15.8±0.68	16.60±0.84	17.8±0.26	18.92±0.65
Total WBC (10^3 /µl)	8.71±0.32	9.75±0.260	10.6±0.27	11.60±1.22
Neutrophils (%)	29.22±0.01	30.02±0.10	31.11±1.12	32.02±1.02
Lymphocyte (%)	58.12±1.32	58.12±1.12	58.10±2.33	58.20±2.62
Monocyte (%)	0.06±0.02	0.06±0.040	0.06±0.010	0.06±0.06
Eosinophil (%)	0.2±0.040	0.20±0.020	0.2±0.010	0.2±0.060
Platelets cells (10^3/µl)	543.14±3.43	543.41±4.12	544.13±4.0	545.12±2.54
Total RBC (10^6/µl)	7.68±0.12	7.76±0.43	7.69±0.48	7.75±0.26
PCV%	49.42±0.2	49.42±1.12	49±1.220	49.60±2.21
MCHC (g/dl)	31.8±1.32	31.24±1.20	32.18±1.10	32.33±1.12
MCV fl (µm^3)	57.3±3.20	57.2±1.20	57.9±1.24	57.8±1.22

*N.S- Not Significant, **(p > 0.01), *(p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)*

Table No.26. Biochemical Parameters of Wistar albino rats group exposed to TVC

BIOCHEMICAL PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
Glucose (R) (mg/dl)	105.14±8.2	105.16±4.10	106.02±11.1	106.12±6.2
T.cholesterol (mg/dl)	108.16±1.42	108.25±1.20	109.62±1.18	109.24±1.63
Triglyceride (mg/dl)	64.16±1.42	64.12±1.22	66.16±1.22	66.16±1.22

BIOCHEMICAL PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
LDL	69.6±2.13	69.12±2.34	69±1.32	69.24±12.12
VLDL	13.4±1.32	13.42±4.24	13.24±2.84	13.54±14.16
HDL	22.16±6.12	22.42±2.20	23.18±2.26	24.18±22.12
Ratio 1 (T.CHO/HDL)	4.61±1.12	4.62±1.24	4.64±1.14	4.64±2.30
Ratio 2 (LDL/HDL)	2.40±1.14	2.41±1.12	2.41±2.20	2.46±10.02
ALBUMIN (g/dl)	4.43±0.16	4.53±0.32	4.44±10.32	4.42±10.48

*NS- Not Significant,**(p > 0.01), * (p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)*

Table No.27. Renal Function Test of Wistar albino rats group exposed to TVC

PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
UREA (mg/dl)	21.30±0.99	21.20±0.36	21.16±1.18	21.48±1.21
CREATININE (mg/dl)	0.42±0.02	0.41±0.04	0.42±0.06	0.44±0.08
BUN(mg/dL)	14.1±0.11	14.10±0.60	14±0.32	14.46±1.12
URICACID (mg/dl)	5.00±0.34	5.06±0.21	5.7±0.14	5.62±0.26

*NS- Not Significant, **(p > 0.01), * (p >0.05) , n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)*

Table No.28. Liver Function Test of Wistar albino rats group exposed to TVC

PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
T.BILIRUBIN (mg/dl)	0.03±0.03	0.03±0.02	0.04±0.02	0.04±0.04
SGOT/AST (U/L)	139.15±1.33	139.34±0.32	140.01±1.62	140.75±1.02
SGPT/ALT (U/L)	72.12±1.18	72.22±1.34	72.14±1.28	72.46±0.61
ALP (U/L)	129.22±3.16	129±12.14	130±14.04	130.23±11.15
T.PROTEIN (g/dl)	8.12±0.34	8.18±0.12	8.16±0.14	8.54±0.49

*NS- Not Significant, **(p > 0.01), * (p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)*

Discussion

- The overall percent of body weight gain in rats treated with the drug was found to be normal and showing a steady increase in weight indicating that the test animals were in a healthy condition during the 28 days of observation period.
- There is no significant change in water intake by the animals during the period of study.
- The weight increase of the animals showed that the intake of food by the animals was good during the period of 28 days study.
- The haematological parameters of animals were done. The results of 28 days oral toxicity study was tabulated above.
- The Blood investigations of RBC, WBC, Hb, Platelets and ESR are normal that is within the limits.
- The differential count, PCV, MCV showed no significant changes.

- Thus the trial drug *Thathu Viruthi Choornam* was good and safe drug for oral administration.

- The biochemical parameters are within the normal range. This shows that the trial drug shows safe and non toxic effects on general body metabolism.

- The renal function test of the animals shows the normal limits thus the trial drug was safe and not produce any nephro toxicity, thus it suggests that the trial drug was safe for long term administration.

- The total bilirubin showed that normal range. Thus the liver function test of *TVC* shows normal in this 28 day repeated oral toxicity.

- According to these results, *Thathu Viruthi Chooranam* could be considered as no-observed-adverse-effect level (NOAEL) drug as it acts harmlessly under the current normal usage and this phenomenon is considered to be of no toxicological concern.

Discussion

Furthermore no dose related histopathological changes were observed. Gross examination in necropsy and at microscopic examination revealed no changes that attribute to the administration of drug.

PHARMACOLOGY STUDIES

Evaluation of Spermatogenic Activity of *Thathu Viruthi Chooranam* in wistar albino male rats by ethanol induced method.

Spermatogenic activity

Decrease in sperm count observed after administration of ethanol was reversed by treating with the study drug *Thathu Viruthi Chooranam*. In the following tables the parameters like Sperm count, Motility, Viability and Morphology of TVC 200 mg /kg treated group shown significant increase than ethanol alone treated group(negative control). In testis histopathological of negative control (group II)animals showed germinal damage and treated group shows significant restoration. From these results it is obvious that *Thathu Viruthi Chooranam* has potent spermatogenic activity.

Table No.29. Effect of TVC on sperm count and motility

Groups	Intervention	Sperm Count	Sperm Motility(%)
Group I[C]	1 ml of Milk	206.12±1.187	86.02±1.83
Group II [IG]	25% of ethanol (0.5 ml/kg/day)	70.69±0.765	26.11±1.67
Group III [TG-1]	TVC (100mg / kg / day) + 25% of ethanol (0.5 ml/kg/day)	120.86±2.51*	62.18±1.783*
Group IV [TG-2]	TVC (200mg / kg / day) + 25% of ethanol (0.5 ml/kg/day)	151.86±1.86**	67.07±1.96**

*Values are expressed as mean ± S.E.M (Dunnett's test). *P<0.05, **P<0.01, ***P<0.001 vs control; N=6*

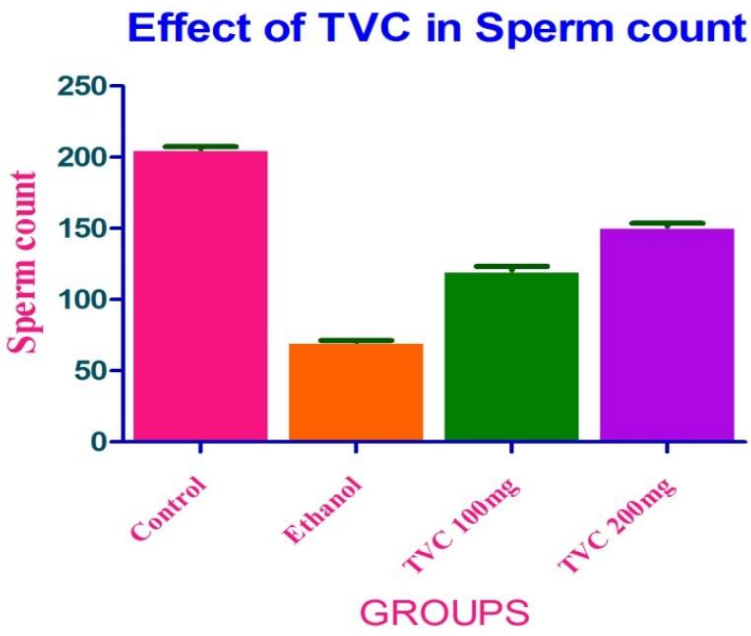


Chart No.1. Effect of TVC on Sperm count

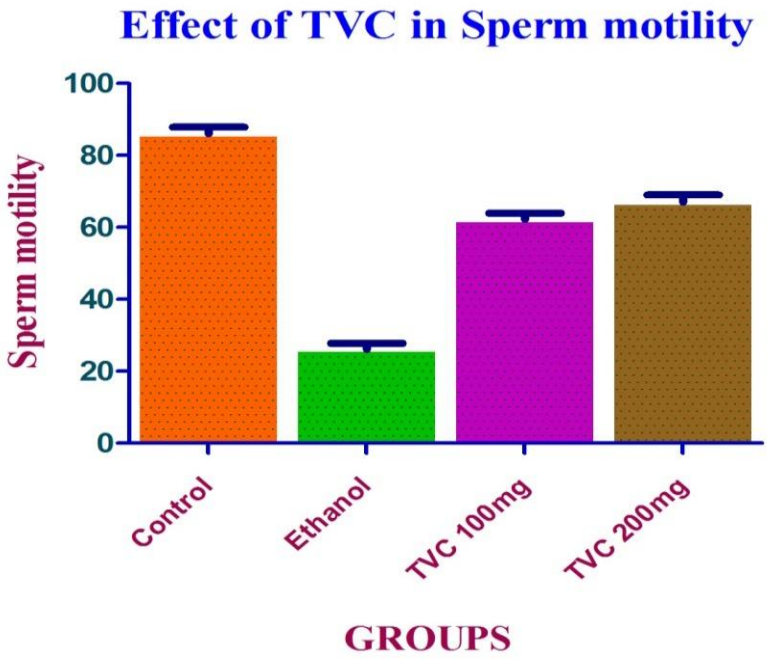


Chart No.2. Effect of TVC on Sperm motility

Table No.30. Effect of TVC on Sperm morphology and viability

Intervention	Morphology		Viability
	Normal	Abnormal	
1 ml of Milk	76.56±4.52	20.72±1.07	68.21±2.66
25% of ethanol (0.5 ml/kg/day)	28.13±0.65	69.59±0.99*	46.61±1.90
TVC (100mg / kg / day) + 25% of ethanol (0.5 ml/kg/day)	70.21±2.78*	23.42±1.03	64.33±2.08*
TVC (200mg / kg / day) + 25% of ethanol (0.5 ml/kg/day)	76.72±2.92**	19.32±0.45	66.63±1.26*

*Values are expressed as mean ± S.E.M (Dunnett's test). *P<0.05, **P<0.01, ***P<0.001 vs control; N=6*

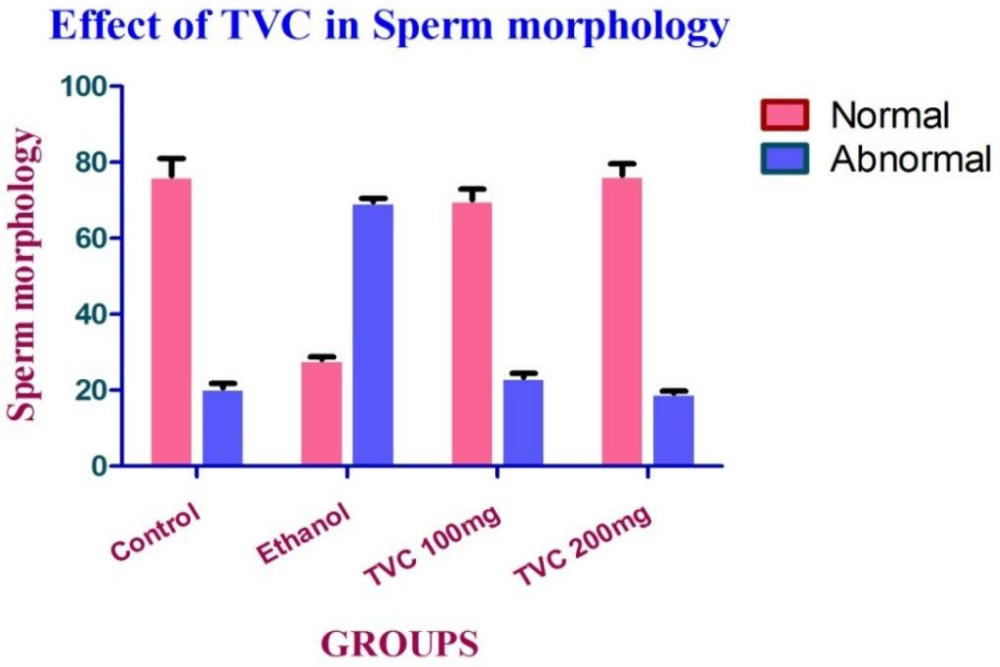


Chart No.3. Effect of TVC on Morphology

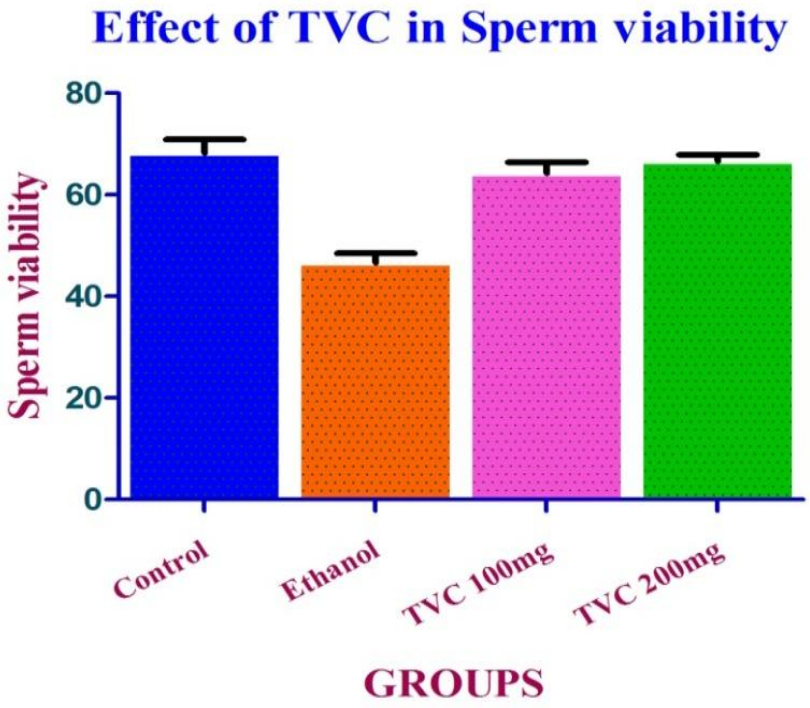


Chart No.4. Effect of TVC on Sperm viability

Table No.31. Effect of TVC on Body and Testis weights

Intervention	Body weight(g)		Testis weight(g)
	1st day	60th day	
1 ml of Milk	195.88±5.02	278.39±3.13	12.16±0.419
25% of ethanol (0.5 ml/kg/day)	196.60±3.25	221.71±1.98	9.563±0.308
TVC (100mg / kg / day) + 25% of ethanol (0.5 ml/kg/day)	199.52±3.90	281.23±3.54	11.25±0.801**
TVC (200mg / kg / day) + 25% of ethanol (0.5 ml/kg/day)	197.64±2.08*	293.62±4.56*	12.46±0.96

*Values are expressed as mean ± S.E.M (Dunnett's test). *P<0.05,**P<0.01,***P<0.001 vs control; N=6*

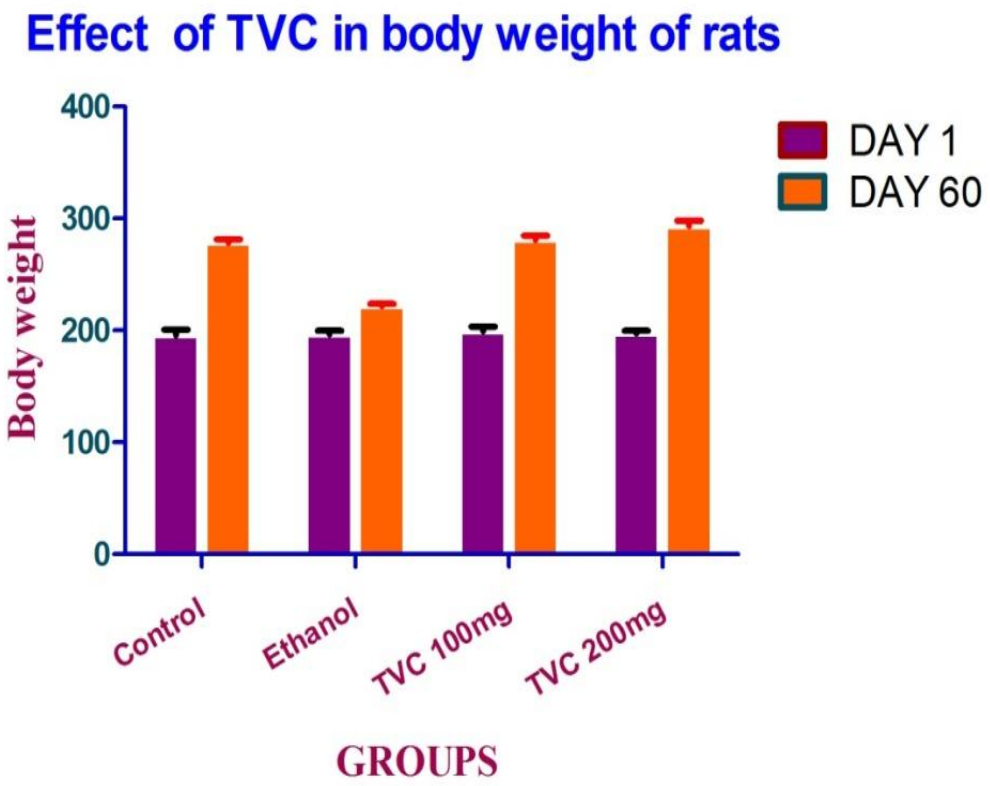


Chart No.5. Effect of TVC on Body weight

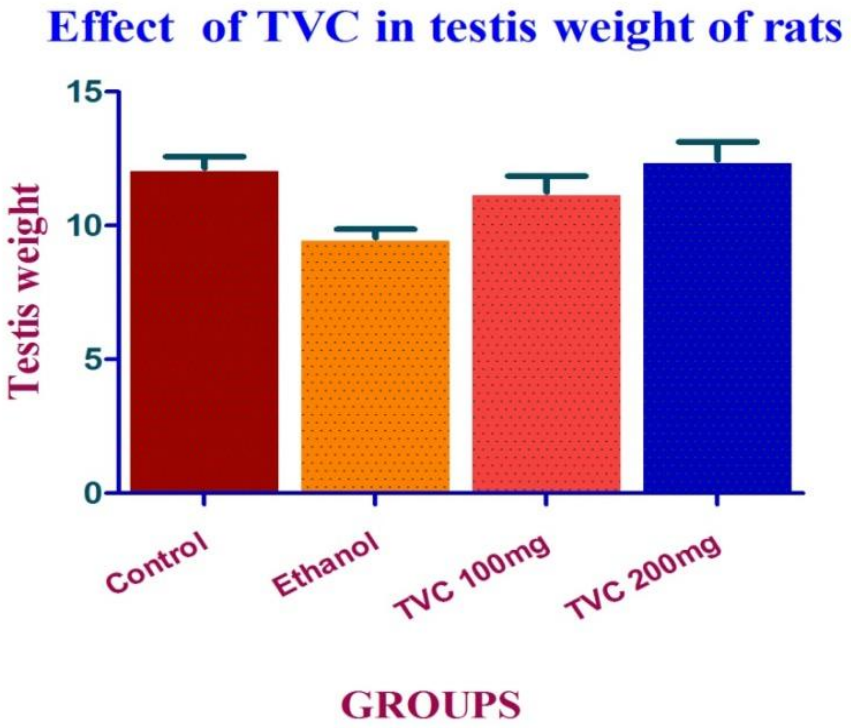


Chart No.6. Effect of TVC on Testis weights

Discussion

From the above obtained results, can conclude that the drug *Thathu Viruthi Chooranam* exhibits the spermatogenic activity by increasing the number of spermatozoa in somniferous tubules. The trial drug possess significant Spermatogenic activity at the dose levels of 100 and 200 mg/kg of body weight. usally plant products play a major role in the functioning of spermatogenesis like ashwagandha. Likewise the drug *Thathu Viruthi Chooranam* also possess potent spermatogenic activity[79]. Already the ingredients of this TVC formulation Curculigo orchioides, mucuna pruriens were proved as a potent drug for spermatogenesis[80,81]. So, this evaluation of spermatogenic activity study will lead the way for the clinical use of the drug.

Evaluation of Aphrodisiac activity of *Thathu Viruthi Chooranam* in Ethanol treated male rats.

Table No.32. Mount frequency observed in the evaluation of Aphrodisiac activity of TVC

Groups	Mount frequency (Sec)
Control	5.26±0.32
Ethanol treated rats	0.66±0.15
Ethanol+ *Thathu Viruthi Chooranam* 100mg/kg	2.54±0.46
Ethanol+*Thathu Viruthi Chooranam* 200mg/kg	4.63±0.14**

*Values are expressed as mean ± S.E.M (Dunnett's test). *P<0.05, **P<0.01, ***P<0.001 vs control; N=6*

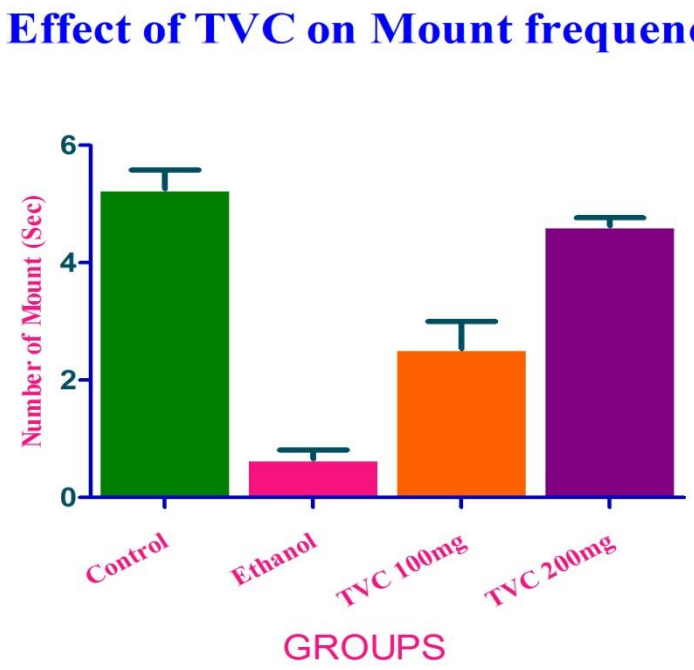


Chart No.7. Effect of TVC on Mount frequency

Table No.33. Intromission frequency observed in the evaluation of Aphrodisiac activity of TVC

Groups	Intromission frequency (Sec)
Control	2.38± 0.16
Ethanol treated rats	0.36±0.09
Ethanol+ *Thathu Viruthi Chooranam* 100mg/kg	1.54±0.12*
Ethanol+*Thathu Viruthi Chooranam* 200mg/kg	2.29±0.22**

*Values are expressed as mean ± S.E.M (Dunnett's test). *P<0.05, **P<0.01, ***P<0.001 vs control; N=6*

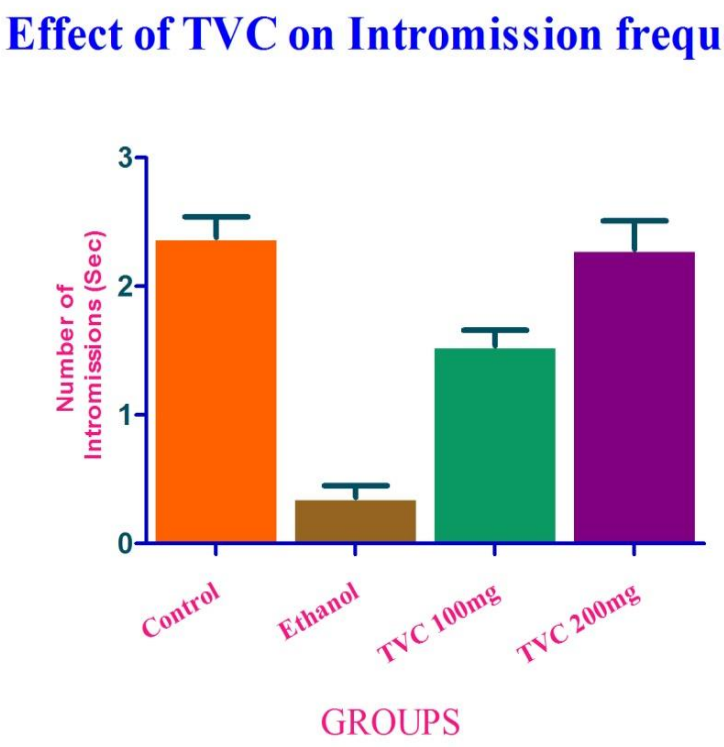


Chart No.8. Effect of TVC on Intromission frequency

Table No.34. Mount latency observed in the evaluation of Aphrodisiac activity of TVC

Groups	Mount latency (sec)
Control	244.21±7.16
Ethanol treated rats	379.14±8.15
Ethanol+ *Thathu Viruthi Chooranam* 100mg/kg	276.19±5.22*
Ethanol+*Thathu Viruthi Chooranam* 200mg/kg	242.00±2.12***

*Values are expressed as mean ± S.E.M (Dunnett's test). *P<0.05, **P<0.01, ***P<0.001 vs control; N=6*

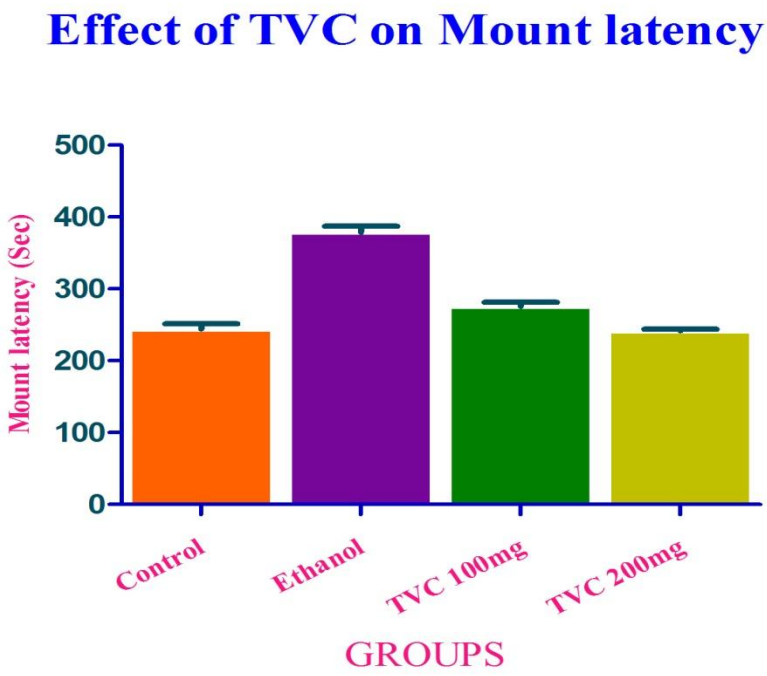


Chart No. 9. Effect of TVC on Mount latency

Table No.35. Intromission latency observed in the evaluation of Aphrodisiac activity of TVC

Groups	Intromission latency (sec)
Control	247.18±206.06
Ethanol treated rats	986.26±328.64
Ethanol+ *Thathu Viruthi Chooranam* 100mg/kg	314.16±200.44**
Ethanol+*Thathu Viruthi Chooranam* 200mg/kg	212.32±158.15***

*Values are expressed as mean ± S.E.M (Dunnett's test). *P<0.05, **P<0.01, ***P<0.001 vs control; N=6*

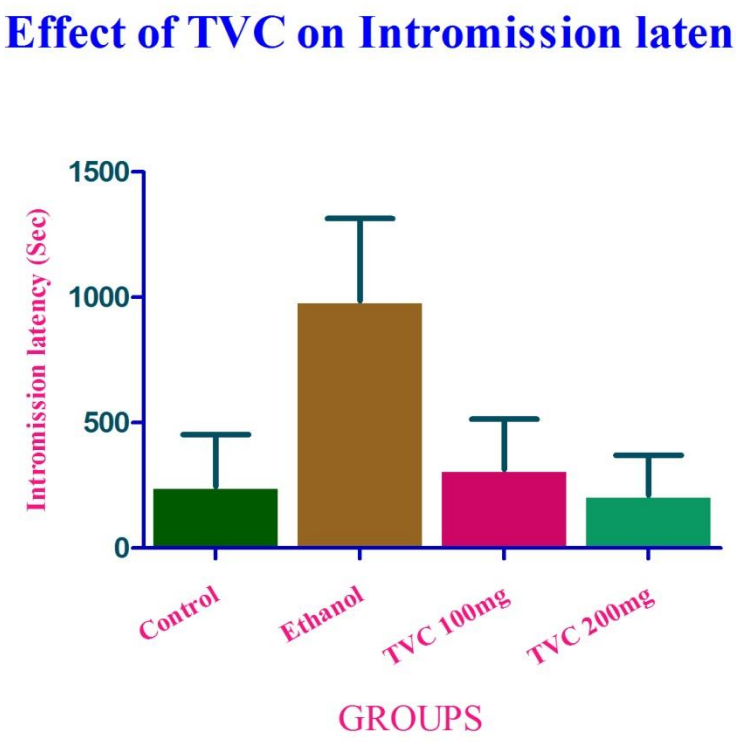


Chart No.10. Effect of TVC on Intromission latency

Table No.36 Ejaculation latency observed in the evaluation of Aphrodisiac activity of TVC

Groups	Ejaculation latency (min)
Control	3.09±0.58
Ethanol treated rats	6.12±0.69
Ethanol+ *Thathu Viruthi Chooranam* 100mg/kg	4.43±0.56**
Ethanol+*Thathu Viruthi Chooranam* 200mg/kg	1.98±0.85***

*Values are expressed as mean ± S.E.M (Dunnett's test). *P<0.05, **P<0.01, ***P<0.001 vs control; N=6*

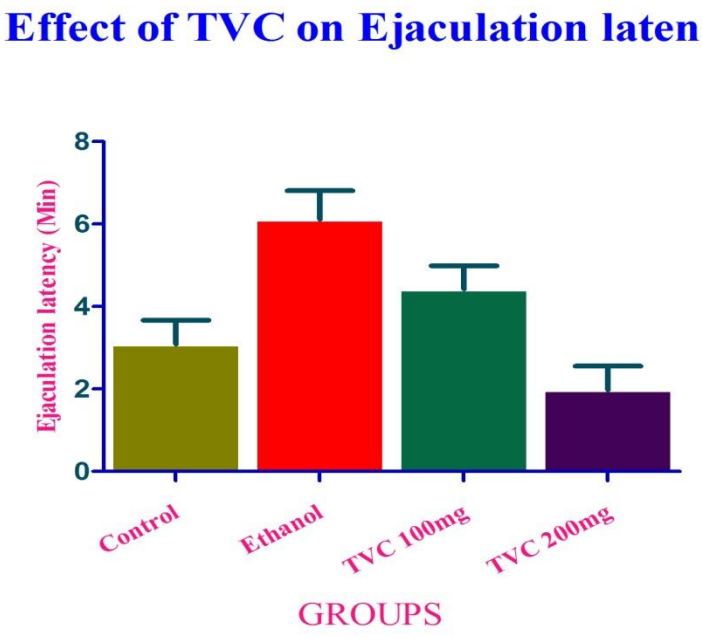


Chart No.11. Effect of TVC on Ejaculation latency

Table No: 37 Post Ejaculatory Interval observed in the evaluation of Aphrodisiac activity of TVC

Groups	Post Ejaculatory Interval (min)
Control	4.22±0.77
Ethanol treated rats	12.34±1.07
Ethanol+ *Thathu Viruthi Chooranam* 100mg/kg	7.67±0.15**
Ethanol+*Thathu Viruthi Chooranam* 200mg/kg	3.02±0.42***

*Values are expressed as mean ± S.E.M (Dunnett's test). *P<0.05, **P<0.01, ***P<0.001 vs control; N=6*

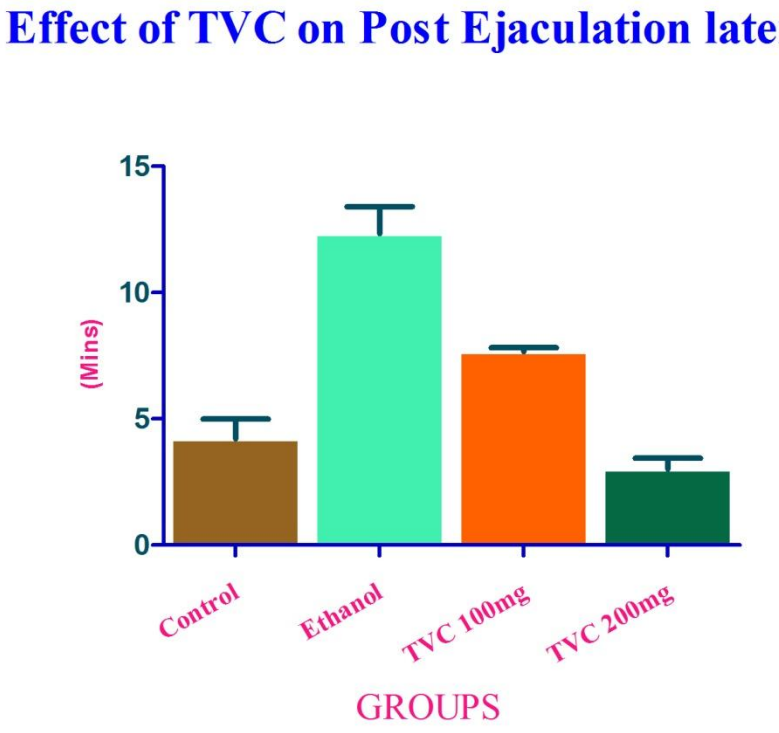


Chart No.12. Effect of TVC on Post Ejaculation Interval

Discussion

From the observed results of mount frequency, intromission frequency, mount latency, intromission of latency, ejaculation latency and post ejaculation latency can confirmed that the trial drug *Thathu Viruthi Chooranam* has the property of Aphrodisiac. The study concluded that the cumulative dose of *Thathu Viruthi Chooranam* could enhance overall sexual function and performance in male rats by increasing the spermatozoa concentration and hormonal levels such as FSH, Testosterone, LH. The drug *Thathu Viruthi Chooranam* showed more potent aphrodisiac activity at the dose level of 200mg/Kg BW and 400mg/Kg BW. The results suggest that the prepared *Thathu Viruthi Chooranam* may be a new promising aphrodisiac combination, which can be used to improve the sex life of many troubled men. Already the scientific literatures are available regarding the evaluation of aphrodisiac nature of herbals[82]. This aphrodisiac property may be due to possible synergistic action of selected plants used in this *Thathu Viruthi Chooranam*.

Evaluation of the anti oxidant activity of *Thathu Viruthi Chooranam* by DPPH Scavenging Assay

Table No.38. Anti-oxidant activity of TVC

Sample concentration (µg/ml)	Absorbance		Percentage of Inhibition	
	Drug	Standard	Drug	Standard
Control	0.5461	0.324	-	-
1.25	0.4632	0.252	15.1803	22.22
2.50	0.3672	0.192	32.7595	40.74
5	0.2430	0.124	55.5026*	61.72**
10	0.1852	0.092	66.0867	71.60
20	0.1012	0.046	81.4685	85.80

µg/ml: microgram per millilitre. Drug: TVC (1.25-20µg/ml). Standard: Ascorbic acid (10mg/ml DMSO)

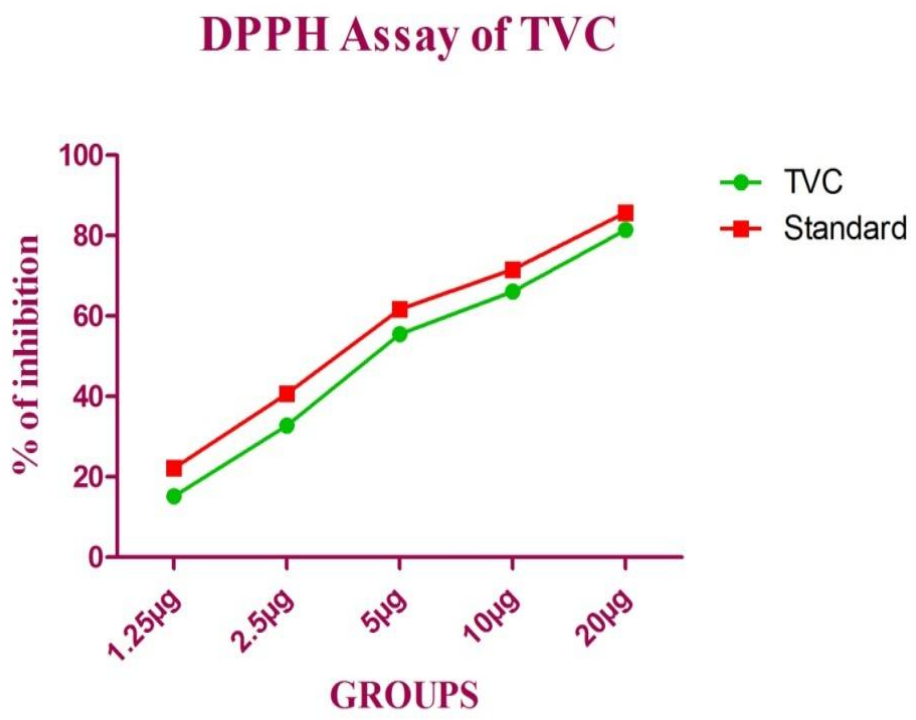


Chart No.13. Antioxidant Activity of TVC

Discussion

From the investigation of DPPH radical scavenging assay of TVC it was concluded that the test drug has shown promising antioxidant activity and exhibits significant percentage inhibition against DPPH radicals when compared to that of standard BHT. Because of this high antioxidant therapeutic nature the drug *Thathu Viruthi Chooranam* will helps to treat the male infertility. Antioxidants play a major role in the treatment of male infertility[83-86]. So the presence of antioxidant property of TVC will be highly useful for the treatment of male infertility.

HISTOPATHOLOGY-28 DAYS REPEATED ORAL TOXICITY STUDY

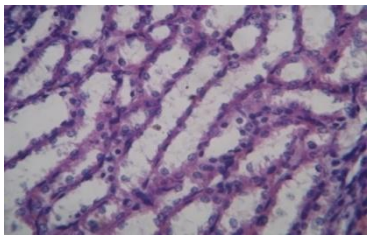
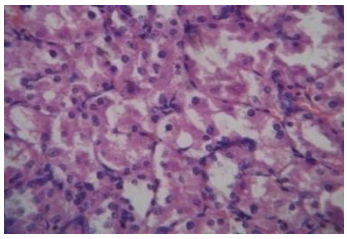
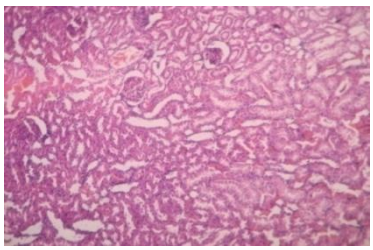
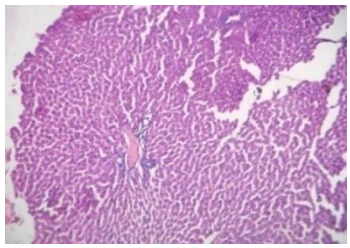
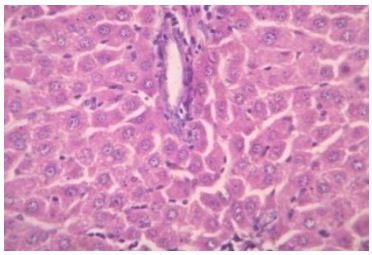
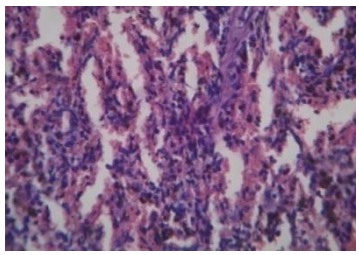
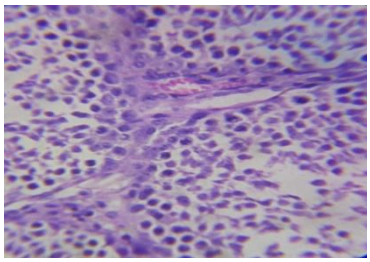
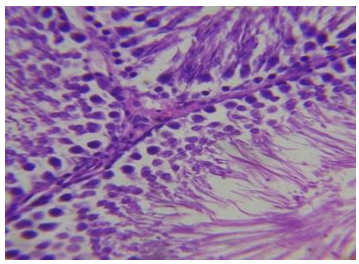
	(100 mg/kg)	(200 mg/kg)
KIDNEY		
LIVER		
SPLEEN		
TESTIS		

Fig No.12. Histopathology image of TVC

CONCLUSION

6. CONCLUSION

- The drug "*Thathu Viruthi Chooranam*" was prepared as per the classical Siddha literature *Sarabenthirar Vaithiya Rathnavali*.
- It fulfills all the standardization parameters of *Chooranam* as mentioned in AYUSH guidelines.
- The results of biochemical analysis showed the presence of acid and basic radicals in the sample.
- The presence of alkaloids, flavonoids, glycosides, tannins, triterpenoids were identified in the choloroform and methanolic extract of *Thathu Viruthi Chooranam* through Phytochemical analysis.
- TLC plate showed different colour phytoconstituents of chloroform extract of TVC.
- FT-IR Study results exhibits the presence of some organic functional groups such as alcohols, phenols, alkenes, amines, aromatics, aliphatic amines, alkyl halides.
- SEM analysis showed the objects of sizes ranging from 3µm to5 µm.
- XRD pattern of the trial drug *Thathu Viruthi Chooranam* shows good crystalinity.
- Based on OECD 423 the trial drug *Thathu Viruthi Chooranam* is considered as non toxic up to the dose of 2000mg/kg.
- The drug *Thathu Viruthi Chooranam* possess the potent Aphrodisiac activity in ethanol treated male rats.
- The drug *Thathu Viruthi Chooranam* could be confirmed as no-observed-adverse-effect level (NOAEL) drug as it acts harmlessly under the current normal usage and this phenomenon is considered to be of no toxicological concern.
- High antioxidant therapeutic nature was evaluated in the drug *Thathu Viruthi Chooranam* through DPPH Scavenging assay. Because of this anti-oxidant property, it can be used as a drug to treat the male infertility.
- Hence it is proved that the drug *Thathu Viruthi Chooranam* was pharamacollogicaly evaluated for the property of Spermatogenic, Aphrodisiac and Antioxidant activity.

SUMMARY

7. SUMMARY

Herbal preparations in Siddha system of medicine always have a unique range of beneficial effect because of its wonderful therapeutic value without causing adverse effects. Here the drug *Thathu Viruthi Chooranam* was pharmacologically evaluated and scientifically validated and standardized.

The ingredients of the drug was identified and authenticated by *Gunapadam* experts. The drug was prepared as per classical Siddha literary procedure and subjected to various studies to reveal its potency and efficacy of the drug.

The organoleptic character and physicochemical studies were made into standardization of the drug TVC. From the above studies the TVC is standardized as per AYUSH guidelines.

The biochemical and instrumental analysis was made to know the presence of active ingredients in the drug which is responsible for its activity.

Here, the biochemical analysis showed the presence of Calcium, Magnesium, Zinc, Sulphate, Chloride by its synergistic effects, the drug as activity against the disease.

In instrumental analysis, FTIR showed the OH group has higher potential towards inhibitory activity against microorganisms. Sometimes the presence of Phenols in medicinal plants possess highly Anti-Oxidant property which enhances the drug effect against the disease.

SEM picture explained the particle size of the drug. In ICP-OES described about the absence of heavy metals and its permissible limits which showed the safety of the drug.

TLC plate showed different colour phytoconstituents of chloroform extract of TVC. The bands revealed presence of seven violets, four reds, two greens, one blue, one white and one yellow showing the presence of alkaloids, glycosides, phenols, triterpenes, flavonoids and quinines.

The drug *Thathu Viruthi Chooranam* was proved that it is free from toxicity through the acute and 28 days repeated oral toxicity study as per the OECD guidelines. In acute toxicity study there was no mortality of rats observed. In 28 days repeated oral toxicity study, the obtained results of haematological, biochemical,

urinary parameters were normal. The histopathological findings did not show any abnormalities.

And this herbal formulation possess more potent Aphrodisiac activity in ethanol treated male rats. This trial drug *Thathu Viruthi Chooranam* exhibits high range of Spermatogenic activity in ethanol induced male rats.

Most of the research findings already reported that the role of antioxidant is essential to treat male infertility. Here the trial drug possess strong antioxidant activity.

These results conclude that the drug *Thathu Viruthi Chooranam* is a best drug of choice for the treatment of male infertility.

By analyzing all those findings, it is proved that the drug *Thathu Viruthi Chooranam* has high range of therapeutic value and the safety of the drug for clinical use is ensured. So, it is confirmed that the herbal formulation *Thathu Viruthi Chooranam* may never cause any adverse effects in clinical use. The preparation of the drug is cost effective too.

So, the clinical trials have to be followed on the drug *Thathu Viruthi Chooranam*. Thus can treat the infertile males effectively and help them to achieve their dream of producing their own offspring.

FUTURE SCOPE

FUTURE SCOPE

Trial drug ***THATHU VIRUTHI CHOORANAM,*** was taken from the classic Siddha Literature ***Sarabenthirar Vaithiya Rathnavali,*** written by **A. Krishnasami Maadikrao Shakeb.** Its validation for Spermatogenic and Aphrodisiac activities were completed at preliminary level. The result enhanced and assured its Spermatogenic and Aphrodisiac activities against male infertility. More specific experiments on animal models and also clinical trials are required to understand the exact molecular mechanism of action. So it could be used worldwide in safe treatment of male infertility.

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ANNEXURE

The Tamil Nadu Dr.M.G.R.Medical University

69,Anna Salai,Guindy,Chennai - 600 032.

This Certificate is awarded to Dr/Mr/Mrs....D..Mahendran.....

for participating as ~~Resource~~ ~~Person~~ / Delegate in the Eighteenth Workshop on

" RESEARCH METHODOLOGY & BIOSTATISTICS "

FOR AYUSH POST GRADUATES & RESEARCHERS

Organized by the Department of Siddha

The Tamil Nadu Dr. M.G.R. Medical University from 20 th to 24 th July 2015.

Dr.N.KABILAN, M.D.(Siddha)
READER,DEPT.OF SIDDHA

Prof. **Dr.P.ARUMUGAM,** M.D.,
REGISTRAR i/c

Prof. **Dr.D.SHANTHARAM,** M.D., D.Diab.,
VICE - CHANCELLOR

C.L.BAID METHA COLLEGE OF PHARMACY

(An ISO 9001-2000 certified institute)

Jyothi Nagar, Old Mahabalipuram Road

Thoraipakkam, Chennai – 600 097

CERTIFICATE

This is to certify that the project entitled, **Toxicological and Pharmacological study on THATHU VIRUTHI CHOORANAM & SIVAKARANDHAI *(Sphaeranthus amaranthoides)* CHOORANAM** in rats pigs submitted in partial fulfilment for the degree of **M.D. (siddha)** was carried out at C.L. Baid Metha college of Pharmacy, Chennai-97, in the Department of Pharmacology during the academic year of 2016-2017. It has been approved by the **IAEC No: IAEC/XLVIII/10/CLBMCP/2016**

C.L. Baid Metha College of Pharmacy ★ CHENNAI-97 ★ Thoraipakkam

(Dr.P.Muralidharan)

IAEC Member Secretary

C.L. BAID METHA COLLEGE OF PHARMACY,
THORAIPAKKAM, CHENNAI - 600 097.

சித்த மருத்துவ மைய ஆராய்ச்சி நிலையம், சென்னை - 600 106
सिद्ध केंद्रीय अनुसन्धान संस्थान,
अण्णा सरकारी अस्पताल परिसर, अरुम्बाक्कम, चेन्नई - 600 106
SIDDHA CENTRAL RESEARCH INSTITUTE
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Phone: 044-2621 4925, Fax: 044-2621 4809

20.1.2017

CERTIFICATE

Name of the student: Dr. D. Mahendran, III year PG student, Gunapadam, Government Siddha Medical College, Arumbakkam, Chennai-600 106.

Name of the sample: Thathu Viruthi Chooranam

Name of the Experiment	I	II	Mean
Loss on drying(at 105°C)	7.52 %	7.80 %	7.66 %
Total ash	7.92 %	8.05 %	7.985 %
Water soluble ash	1.49 %	1.88 %	1.685 %
Acid insoluble ash	1.94 %	1.69 %	1.82 %
Water soluble extractive	31.0 %	32.4 %	31.7 %
Alcohol soluble extractive	27.6 %	29.6 %	28.60 %
pH value (10%)	5.58	5.59	5.58
TLC/HPTLC	Report Enclosed		

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Research Officer (Chemistry) & Head,
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Research Officer (Scientist II) (Siddha)
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